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*THE GENETIC DEMONSTRATION OF DOUBLE STRAND CROSSING-OVER IN ZEA MAYS**

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Conclusive evidence has been obtained in the genetical studies of Bridges and Anderson[1] and Anderson[2] that the X chromosome of Drosophila melanogaster undergoes crossing-over at a stage in which each chromosome is split into two strands and that at any one point (chiasma) only two of the four strands exchange parts. Redfield[3] and Dobzhansky and Sturtevant[4] have likewise shown that when the second and third chromosomes of Drosophila cross over they do so in a double strand stage. Unfortunately the cytological studies of oögenesis in Drosophila females have failed, owing to the difficulty with which clear figures are obtained, to shed any light on the mechanism through which the crossing-over occurred.

Recently a number of investigators, notably Belling, Darlington and Sax, working with plant material have, on the basis of their cytological observations, presented various theories to explain, by a cytological mechanism, the genetic phenomenon of crossing-over. While there is disagreement between the various theories as to how the actual exchange of pieces of chromatin takes place yet they agree with the genetically determined facts in Drosophila that crossing-over occurs at a double strand stage and that only 2 of the 4 chromatids exchange parts at any one chiasma. These cytological observations on plant sporocytes parallel in a remarkable way the genetic data for Drosophila. But in none of the plants used in the cytological studies, nor in any plant, has it been possible to demonstrate the occurrence of double strand crossing-over by genetical means. There exist certain data in the literature, Blakeslee, et al,[5] Allen,[6] Frost,[7] which can be interpreted as a result of double strand crossing-over but other equally plausible interpretations are possible. The purpose of this paper is to present data for the genetical proof of double strand crossing-over in Zea mays.

The tetrad present at the first metaphase in meiosis of diploid organisms consists of 4 chromatids, two of which are derived by the splitting of one

parental chromosome at some time previous to or at the time of crossing-over and the other two chromatids arise in a similar manner from the other parental chromosome. Normally the 4 members of the tetrad are distributed in the two meiotic divisions to the quartet of cells arising from each meiocyte so that each member of the quartet has only 1 of the 4 chromatids originally present in the tetrad. With such regularity of distribution it is impossible to determine in diploid forms whether or not crossing-over takes place at a double strand stage. If, however, through some unusual circumstance, such as non-disjunction or the attachment of two homologous chromosomes to a single spindle fibre, members of the quartet receive more than 1 of the 4 chromatids present at metaphase 1, it is then possible to show by genetical means that the chromosomes were split at the time crossing-over occurred. It was through these aberrancies that double strand crossing-over was demonstrated by Bridges[3] and Anderson[2] for the X chromosome and by Dobzhansky and Sturtevant[4] for the second chromosome in diploid females of Drosophila. In trisomic and polyploid individuals where the number of strands present at metaphase 1 makes it possible for members of the quartet to regularly receive more than one strand it is possible to determine whether or not double strand crossing-over occurs. Redfield[3] working with triploid Drosophila proved that for the third chromosome crossing-over took place in a double strand stage as did Bridges and Anderson[1] for the X chromosome.

In maize the *pr*—v_2 linkage group has been found by McClintock** (unpublished) to be associated with a specific member of the monoploid set of 10 chromosomes. She has succeeded in isolating strains that are trisomic for this chromosome. These *pr*—v_2 trisomic strains were utilized in testing for the occurrence of double strand crossing-over. Trisomic individuals of the constitution $\frac{Pr \quad V_2}{Pr \quad V_2}$ were pollinated by diploid $\frac{pr \quad v_2}{pr \quad v_2}$ plants of $\frac{pr \quad v_2}{pr \quad v_2}$ constitution. (*Pr*-*pr* are purple-red aleurone factors and V_2-v_2 are green versus virescent seedling factors. They are situated about 43 crossover units apart.) The seeds resulting from the backcross were classified for aleurone color. The ratio of purple to red was 3.3:1 in the 1439 seeds from the 3 ears whose progeny were tested this past winter in the greenhouse. The two classes of seeds were planted and the resulting seedlings classified for green and virescent seedling color. The ratio of green to virescent seedlings was 2.9:1 where a 1:1 ratio is expected if disomic plants heterozygous for V_2 are backcrossed.

The virescent seedlings from the purple seeds were closely examined for the occurrence of $2n + 1$ plants. Mature plants trisomic for the pr—v_2 chromosome differ markedly from their disomic sibs in general habit of growth and width of leaves. They can accurately be distinguished from the $2n$ plants. This difference in appearance between the $2n + 1$ and $2n$ individuals manifests itself in the seedling stage and many of the $2n + 1$ plants can be identified while seedlings. The accuracy of seedling classification is not as great as for mature plants so all individuals classified as trisomic by their seedling appearance had their chromosome number checked by counts either in meristematic cells of root tips or in microsporocytes or both. The employment of seedling appearance as a tentative basis for trisomic classification made it possible to examine relatively large populations of seedlings. It is by no means certain that all trisomic plants can be identified by seedling appearance but a majority of them can be. Nine virescent plants from purple seeds were found which were trisomic for the pr—v_2 chromosome. It was possible to self 6 of these 9 individuals and 2:1 ratios for purple-red aleurone were obtained. Therefore, at least 6 and probably all 9 of the $2n + 1$ plants from purple seeds were of the constitution $\frac{Pr \quad v_2}{pr \quad v_2}$ since McClintock and Hill[9]

$$\frac{pr \quad v_2}{pr \quad v_2}$$

found that Rrr plants gave 2:1 ratios for colored-colorless aleurone when self pollinated. The constitution of the eggs giving rise to these 9 plants must have been $\frac{Pr \quad v_2}{pr \quad v_2}$ since the male pronucleus carried a single

$\frac{pr}{} \; v_2$ chromosome. The formation of such gametes is possible only if crossing-over has taken place at a double strand stage and their occurrence is taken to definitely prove that crossing-over between Zea chromosomes occurs at a double strand stage.

No plants of $\frac{Pr \quad v_2}{Pr \quad v_2}$ or $\frac{pr \quad v_2}{pr \quad v_2}$ constitution were found. Their occurrence could be, however, attributed either to non-disjunction or to double strand crossing-over. This is not true for the 6 tested plants or for the 3 which were presumably of a similar constitution. The failure to find $2n + 1$ individuals homozygous for pr is taken to mean that its locus is close to the spindle fibre region. This is in agreement with the data of Rhoades,[10] in part unpublished, on semisterile-4 where the genetical and

cytological data would place *pr* closer to the spindle fibre than v_2. With large numbers of individuals an occasional trisomic plant homozygous for *pr* should be found unless *pr* is very close to the insertion region. It is obvious that the frequency with which $2n + 1$ plants are found homozygous for a recessive factor is a function of the crossover distance of that locus from the spindle region.

Although 9 trisomic plants homozygous for v_2 and none for *pr* were obtained from approximately 1400 seeds it by no means follows that these frequencies of homozygosis are the correct values. Only those seedlings which showed the marked characteristics of trisomic appearance were examined and it is entirely possible that trisomic plants homozygous for v_2 and *pr* were overlooked. The experiment was designed to show only that double strand crossing-over occurred. This summer more adequate data on the frequencies of v_2 and *pr* will be obtained.

Summary.—The occurrence of double strand crossing-over in Zea mays has been genetically demonstrated.

Acknowledgment.—I wish to thank Dr. Barbara McClintock for her generosity in supplying the author with the *pr*—v_2 trisome. I also wish to thank Dr. R. A. Emerson for his aid and suggestions.

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** McClintock[11] has indirect cytological evidence for the occurrence of double strand crossing-over in Zea and Creighton and McClintock will present further (unpublished) cytological evidence of double strand crossing-over in Zea at the Genetics Congress to be held at Ithaca this summer. Zea thus becomes the first organism in which double strand crossing-over has been demonstrated both genetically and cytologically.

[1] Bridges, C. B., and Anderson, E. G., *Genet.*, **10**, 418–441 (1925).

[2] Anderson, E. G., *Ibid.*, **10**, 403–417 (1925).

[3] Redfield, Helen, *Ibid.*, **15**, 205–252 (1930).

[4] Sturtevant, A. H., and Dobzhansky, T., Carnegie Inst. of Washington (1931).

[5] Blakeslee, A. F., Belling, John, and Farnham, M. E., *Bot. Gaz.*, **76**, 329–373 (1923).

[6] Allen, Charles E., *Genet.*, **15**, 150–188 (1930).

[7] Frost, Howard B., *Proc. Nat. Acad. Sci.*, **17**, 434–436 (1931).

[8] Bridges, C. B., *Genet.*, **1**, 1–52, 107–163 (1916).

[9] McClintock, Barbara, and Hill, Henry, *Genet.*, **16**, 175–190 (1931).

[10] Rhoades, M. M., *Proc. Nat. Acad. Sci.*, **17**, 694–698 (1931).

[11] McClintock, Barbara, *Mo. Agr. Exp. Sta. Bulletin*, **163** (1931).

THE BEHAVIOR OF VERMILION-SUPPRESSOR IN MOSAICS

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It is a point of interest whether the suppressors of the effects of mutant genes affect development in the same way as do the wild-type allelomorphs of these genes, or behave in some other manner. The case of vermilion-suppressor in *Drosophila melanogaster* presents the advantage for the study of this problem that the suppressor is known to be a recessive mutant gene at another locus, and not a duplicating wild-type allelomorph of vermilion (Schultz and Bridges, 1932).

The gene vermilion has the peculiarity (Sturtevant, 1920) that, in mosaics, not only the genetic constitution of the eye, but also that of the rest of the fly, determines the color of the mosaic patch. Tissue that is genetically wild type in such a mosaic, however, is "self-differentiating;" its characteristics are determined, as Sturtevant points out, by its own genetic constitution. The behavior of vermilion-suppressor in mosaics therefore provides a test of the similarity of its behavior to that of the wild-type allelomorph of vermilion.

Two series of experiments were carried out. In one set, males containing the genes vermilion-suppressor, apricot, crossveinless, vermilion and forked, were made heterozygous for a duplication covering the suppressor. This duplication (134), found in an x-ray experiment by Dr. Th. Dobzhansky, belongs to the group of eversporting chromosome rearrangements, and thus frequently gives mosaic patches. In the presence of Duplication 134 the color of the eyes of the flies in question was apricot vermilion. Those cells from which this duplication was lost, however, are genetically vermilion-suppressor as well as apricot vermilion, and whole flies of this type are apricot suppressed-vermilion (brown instead of lemon-yellow). In a series of about a thousand such males, no apricot spots (i.e., with vermilion suppressed) were found. This evidence was not regarded as entirely conclusive, however, since Duplication 134 gives quite small patches, and since in these experiments there could be no independent method of checking the genetic constitution of a given piece of tissue.

Accordingly, females of the constitution vermilion Minute-*n*/vermilion-suppressor apricot crossveinless vermilion forked were synthesized. The Minute-*n* chromosome is frequently eliminated (Bridges, 1925), so females carrying this chromosome often show mosaic patches in which only the not-Minute-*n* sex chromosome remains. As Bridges has shown, in these

Minute-*n* mosaics, vermilion behaves just as in ordinary gynandromorphs, that is, may fail to show in tissue genetically vermilion. In the present case, the patches are of the genetic constitution vermilion-suppressor apricot crossveinless vermilion forked. The characters apricot and forked serve as aids to the detection of such spots in the head; in the mosaic a few apricot ommatidia are clearly distinguishable in the vermilion eye, as are the forked bristles from their Minute neighbors. Moreover, in the larger patches occasionally occurring, the characteristic apricot color is clearly distinguishable from the lemon-yellow of the apricot-vermilion combination. The ability to make this distinction is crucial; for if the action of vermilion-suppressor is dependent on factors outside the cell which is the site of reaction, the mosaic tissue should appear apricot vermilion. But if it behaves like the normal allelomorph of vermilion, that is, if the occurrence of its particular chain of reactions depends on the genetic constitution of the cell involved, such mosaic patches should be apricot, not apricot vermilion.

Fifteen individuals with mosaic eyes were obtained. In thirteen of these the patches were definitely apricot vermilion, four involving from one-fourth to one-half the eye. The two remaining cases were doubtful, since very small patches were concerned. It is clear that the suppressor of vermilion shows the same dependence on factors outside the mosaic tissue as does vermilion, and is not autonomous in its developmental effects, thus differing from the normal allelomorph of vermilion.

This fact indicates that the suppressor operates on a stage of the reaction other than that at which the difference between wild type and vermilion is established. It is interesting in this connection that the color changes in the pupal development of the eye of flies in which vermilion is suppressed resemble those of the wild type, not those of vermilion. The effect of the suppressor must therefore be exerted before the onset of pigment formation.

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STRATIGRAPHICAL RESEARCH IN CENTRAL MEXICO

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The establishment of culture sequence is one of the fundamental aspects of archaeology. Beginning with the earliest discovered cultures in the Valley of Mexico, the American Museum of Natural History has devoted five digging seasons to that end, under the direction of the writer, assisted by his wife, and under the inspection of the Department of Historic Monuments of the Ministry of Public Education of the Mexican Government. During the winter of 1931–32, the focal point of our study was the relationship of the culture of San Juan Teotihuacan, sometimes known as Toltec, and the so-called "archaic" cultures of Zacatenco[1] and Ticoman.[2]

The work fell into four phases:

1. Analysis of depositions at Group 5′ west of the Pyramid of the Moon at San Juan Teotihuacan.

2. Analysis of an early site at Gualupita, Cuernavaca, Morelos.

3. Study of a culture found at San Francisco Mazapan adjacent to Teotihuacan.

4. The sinking of pits at various points around San Juan Teotihuacan and the running of shafts into the Pyramids of the Sun and Moon.

Although the final results are not yet fully digested and correlated, certain salient points appear.

At Group 5′ at Teotihuacan a careful study was made of the terrain to ascertain whether or not heaps of rubbish existed which were susceptible of stratigraphical dissection. It was found that such heaps did not exist in the vicinity of monuments, and whatever accumulations that may have been formed were utilized to provide adobe bricks for buildings or as foundation material for floors. However, three ceramic periods seemed to be discernible.

An early phase (characterized by the predominance of painted over incised decoration) was found in the adobes of the main precinct wall of the group. A middle phase occurred in the foundations for floors outside of and doubtless later than the original precinct. This pottery showed a strong emphasis on incised decoration, and black ware was extensively utilized. The third and latest phase was found above all floors. Gray wares tended to replace the black, and the so-called portrait type of figurine was common.

The Gualupita work, the second phase of our investigation, yielded excellent results, three periods being found. First an early period showing

figurine heads associable although not identical with early periods at Zacatenco and El Arbolillo. This material was contained in a sort of platform or revetment against which had accumulated debris of a later period, containing sherds similar to those found in Ticoman. Yet one or two sherds occurred that seem to have emanated from Teotihuacan. Resting above these deposits was a layer of pottery which contained a polished red and a polychrome ware distinctive of Aztec times, but which lacked the characteristic black on orange of the Valley of Mexico Aztec. In the bottom strata of Gualupita a number of burials were found, the bones of which had almost disintegrated, but which had pots and figurines as funeral furniture. Moreover, in the 150 burials of the early cultures opened by us in the Valley of Mexico, figurines were never buried with the dead. At Gualupita, then, there were three periods, an early one connecting with Early and Middle Zacatenco, a middle which had cultural connections with Ticoman but was chronologically tied in with Teotihuacan and a late period which, although closely affiliated with Aztec, lacked the characteristic culture diagnostic of black on orange pottery.

The third phase of the digging brought us back to San Juan Teotihuacan. A farmer living in San Francisco Mazapan had discovered on his property a burial richly equipped with pottery vessels. Further exploration by us revealed a series of nine skulls, each in a bowl with another inverted over it, but the body bones of only one individual were found. Some eighteen caches of pots were also uncovered and finally two burials with a lavish offering of pottery vessels were exhumed. Two of these bowls were very finely executed portrait vases. This ceramic upon analysis was not "archaic," nor was it Teotihuacan or Aztec. But, as Aztec sherds were found in the top soil, and as Mazapan debris was found overlying Teotihuacan material at the pyramid site, this Mazapan culture may be assigned with some confidence to a post-Teotihuacan, but pre-Aztec date. The material is significant as filling in that gap in the history of the Valley sometimes referred to as the Chichimec period.

The fourth phase of our digging, comprising the sinking of pits at San Juan Teotihuacan, tended to confirm our suspicions as to the sequence of types. The ceramic material found in the pyramids of the Sun and Moon showed a predominance of painted pottery over incised. This pottery was by no means identical with or derived from the early culture sites of Zacatenco, Ticoman and El Arbolillo, although it might be called "Archaic Teotihuacan." In fact, indications at Gualupita and at Ticoman as well, strongly suggest partial contemporaneity between Teotihuacan and the latter phases of the Early Cultures. Other pits confirmed our estimate that the black and carved wares gave way to rising proportions of gray ware in the upper cuts.

In the town of San Sebastian a pit was dug in five equal layers. The bottom and fifth stratum showed a mixture of Mazapan and late Teotihuacan sherds. The next two layers produced Mazapan sherds with the addition of Aztec polished red, but the two top layers revealed this polished red associated with the regulation Aztec black on orange. Thus the resemblance of the third and fourth cuts of San Sebastian to the top layer of Gualupita in that black and orange is absent while the polished red wares are most common, may mean that the former ware is associable with the Tenochtitlan Aztecs and may therefore serve as a diagnostic in tracing their domination over the other Nahua tribes in the Valley.

The salient results of the season may be said to consist of finding local

CERAMIC SEQUENCE IN THE VALLEY OF MEXICO

Tenochtitlan (A)	Tenayuca (A)[3] Middle (?) Tenayuca (A)	Texcoco (A) Acolhua? (A)	Teopanzalco (A) Gualupita III (A)
San Juanico III (M) El Arbolillo III (M-T)	Coyotlatelco (T)	Mazapan (M)	
Cuicuilco (E)	Sub-Tenayuca (E?) Late Ticoman (E) Middle Ticoman (E) Early Ticoman- Late Zacatenco (E)	Late Teotihuacan (T) Middle Teotihuacan (T) Early Teotihuacan (T)	Gualupita II (E)
San Juanico II (E) Copilco (E)	Middle Zacatenco- El Arbolillo II (E)		
San Juanico I (E)	Early Zacatenco- El Arbolillo I (E)		Gualupita I (E)
Undiscovered stages culturally equivalent to Basket Maker I—Pueblo IV			

variations of the Early Cultures at Cuernavaca, establishing the fact that the Teotihuacan culture was not derived from Valley groups of the Early periods, arranging a ceramic sequence of three periods for Teotihuacan and thereby dating as posterior the site of Coyotlatelco near Azcapotzalco, defining the Mazapan culture and dating it as post-Teotihuacan and pre-Aztec, and lastly, finding a suggestive means of distinguishing Tenochtitlan Aztec remains from those of their predecessors like the Acolhuas, the Tlahuicas, the Tepanecas, etc.

The ceramic sequence of the Valley of Mexico is expressed herewith in tabular form. Although further research will expand and change many of the details, the salient points will probably stand the rigid criticism of future study. The data will be published in full in the Anthropological

Papers of the American Museum of Natural History. The qualitative grouping of the sites is expressed by letters, A—Aztec, M—Mazapan, T—Teotihuacan, E—early or "archaic" cultures.

[1] Vaillant, George C., "Excavations at Zacatenco," *Anthropological Papers of the American Museum of Natural History*, **32**, part 1, New York (1930).

[2] Vaillant, George C., "Excavations at Ticoman," *Anthropological Papers of the American Museum of Natural History*, **32**, part 2, New York (1931).

[3] A comprehensive study of this site is about to be published by the Department of Historic Monuments of the Mexican Government. It will give a fuller periodization than the above.

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*ISOVIOLURIC ACID (ALLOXAN-6-OXIME)**

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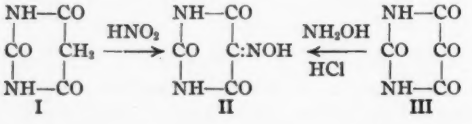
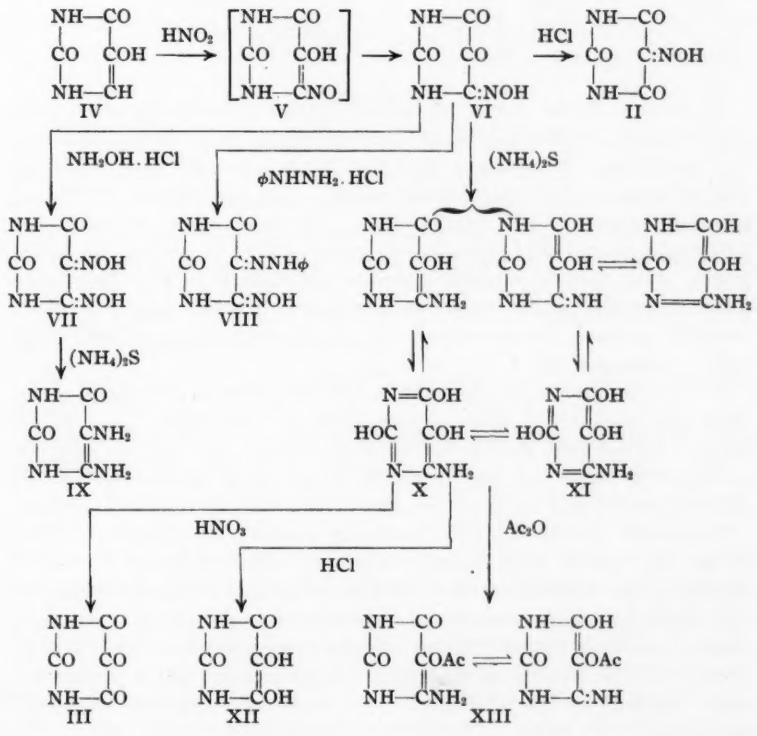
Introduction

Since its discovery by Baeyer[1] violuric acid (II) has been of perennial interest to chemists, largely on account of the beautifully colored salts which it forms. Baeyer prepared this substance by the action of nitrous acid on barbituric acid (I), while Ceresole[2] showed that it could be produced by the action of hydroxylamine hydrochloride on alloxan (III).

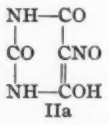


We have found that the action of nitrous acid on isobarbituric acid (IV) leads to the formation of an isomer of violuric acid which we term isovioluric acid (VI). Since the phenolic character of isobarbituric acid has been established by its behavior toward acetic anhydride, hydroxylamine, hypobromous acid,[3] diazomethane[4] and potassium ferricyanide,[5] it was anticipated that 5-hydroxy-6-nitrosouracil (V) would be obtained. The behavior of the product, however, indicated it to be the tautomeric form (VI) (alloxan-6-oxime).

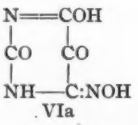
* Contribution from the Chemical Laboratories of Columbia University, with the coöperation of the Research Department of the American Manufacturers of Toilet Articles. The authors gratefully acknowledge financial aid received from the Warren fund of the American Academy of Arts and Sciences.



Properties of Isovioluric Acid.—Although isovioluric acid is lemon-yellow in color as compared with the pale amber of violuric acid, the salts of isovioluric acid are generally orange to red as compared with the much deeper red to blue colors of the violurates. These color relationships may be accounted for by considering the structural formulas involved. In VI we find two adjacent carbonyl groups, while in II the two corresponding carbonyl groups are separated by a third carbon atom. These formulas account for the difference in the depth of color of the two acids. In the case of the salts, on the other hand, we may consider the violurates to be derived from the nitrosophenol[6] form (IIa) of violuric acid, which is colored on account of the NO group present, while in the isoviolurates we may assume that salt formation involves merely the enolization of an NH—CO group to N:COH (VIa).



IIa

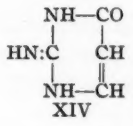


VIa

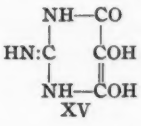
On account of the reactive 5-carbonyl group which it has in common with alloxan, isovioluric acid oxidizes potassium iodide in acid solution, and undergoes condensations with phenylhydrazine and hydroxylamine. The latter reactions yield alloxan-6-oxime-5-phenylhydrazone (VIII) and alloxan-5,6-dioxime (VII), respectively. The dioxime may be reduced to 5,6-diaminouracil (IX),[7] thus allowing a new method of entering the purine group. Like alloxan, isovioluric acid is sensitive to alkali. Thus, with dilute sodium hydroxide it gives an orange-red salt which soon decolorizes, however, probably on account of a rearrangement similar to that of alloxan to alloxanic acid.[8]

Since isovioluric acid represents an unusual oxime of alloxan, it is not surprising to find that dilute hydrochloric acid rearranges it to violuric acid. The simplest mechanism which suggests itself for this reaction involves two steps: (1) the hydrolysis of isovioluric acid to alloxan and hydroxylamine, and (2) the recondensation of these to form violuric acid.

Isouramil.—Isovioluric acid is readily reduced by means of dilute ammonium sulfide to $C_4H_5O_3N_3$. Owing to the benzenoid system of oscillating double bonds present in the completely enolized uracil ring, this substance may be represented by two desmotropic formulas; namely, 5-hydroxy-6-aminouracil (X) and 5,6-dihydroxycytosine(= dialuric acid-4-imine) (XI). Because of its close relationship to uramil we propose the name isouramil for this substance. The properties of isouramil are readily accounted for by means of formula XI, which relates it to dialuric acid. In fact, isouramil is converted into dialuric acid (XII) by means of dilute hydrochloric acid, and, like dialuric acid, it is easily oxidized to alloxan (in this case, with the simultaneous hydrolysis of the amino group). Accordingly, it is a strong reducing agent toward silver nitrate or Fehling's solution. Furthermore, like dialuric acid, it gives a blue color with ferric chloride and ammonia, as well as a purple precipitate with barium hydroxide. The purple precipitate from isouramil is, however, decolorized by excess of the reagent. This behavior distinguishes isouramil from dialuric acid, but is in agreement with the experience of Wheeler and Johnson,[9] who found that isocytosine (XIV), when treated with bromine water and barium hydroxide, gave a blue color which was also discharged by excess of the barium hydroxide. In this case, dialuric acid-2-imine (XV) may be assumed to be an intermediate product in the formation of the colored precipitate.[10] Finally, isouramil is converted into a monoacetyl derivative (XIII) by means of acetic anhydride.



XIV



XV

Experimental

Preparation of Isovioluric Acid (VI).—Six and three-tenths g. (0.05 mol.) of isobarbituric acid[11] was dissolved in 40 cc. of 10% sodium hydroxide (0.10 mol.) together with 3.7 g. of sodium nitrite (0.055 mol.). This solution was then stirred into a mixture of 18 g. (0.3 mol.) of glacial acetic acid and 50 g. of ice. Stirring was continued for half an hour, whereupon the light yellow precipitate was filtered by suction and washed with cold water. Air drying yielded 8.2 g. (85% of theory) of analytically pure isovioluric acid dihydrate.

Anal. Calcd. for $C_4H_3O_4N_3.2H_2O$: N, 21.8; H_2O, 18.7%. Found: N, 22.2; H_2O (110°), 18.0%.

Dehydration at 110° gave bright yellow anhydrous isovioluric acid, which melted at about 250° (dec.).

Anal. Calcd. for $C_4H_3O_4N_3$: C, 30.6; H, 1.9; N, 26.8%. Found: C, 30.5; H, 2.3; N, 26.7%.

Isovioluric acid is insoluble in water and alcohol in the cold, but is readily soluble hot. It dissolves in sodium hydroxide or ammonia to give orange solutions which soon become decolorized. With barium hydroxide, it gives a brick-red precipitate. In the presence of sodium acetate, it gives red colors with Ni^{++}, Co^{++}, Ag^{+}, reddish brown with Fe^{++} and green with Cu^{++}.

Rearrangement of Isovioluric Acid (VI) to Violuric Acid (II).—One and ninety-three-hundredths g. (0.01 mol.) of isovioluric acid dihydrate was suspended in a solution of 2 cc. (0.02 mol.) of concentrated hydrochloric acid in 25 cc. of water. The mixture was heated under reflux in a boiling water bath for four hours. The isovioluric acid quickly went into solution and was replaced by a faint yellow precipitate (alloxan-5,6-dioxime) which gradually redissolved as the heating progressed. On allowing the reaction mixture to stand in the cold, large, clear, pale yellow crystals of violuric acid slowly deposited. Yield, 0.68 g. (43% of theory). By using the mother liquor for the rearrangement of a second batch of isovioluric acid, a yield of 1.10 g. (70% of theory) was obtained. The product was identified by means of its colored salts particularly the deep blue ferrous salt, and by its decomposition at 245–50°.

Alloxan-5,6-dioxime.—One gram of anhydrous isovioluric acid was dissolved in 35 cc. of absolute alcohol and treated with one equivalent of powdered hydroxylamine hydrochloride. The mixture was heated under

reflux for one hour. A precipitate began to separate within a few minutes after heating was begun. The product was filtered, washed with absolute alcohol and dried at 80°. Yield, 1.07 g. (88% of theory) of a faint yellow powder, m. 242° (dec.).

Anal. Calcd. for $C_4H_4O_4N_4 \cdot H_2O$: C, 25.3; H, 3.1; N, 29.5%. Found: C, 25.1; H, 3.1; N, 30.1%.

Alloxan-5,6-dioxime is much less soluble than isovioluric acid, being insoluble even in hot water, alcohol or glacial acetic acid. It does not lose its molecule of water at 110°. It dissolves in alkali and ammonia to give orange-brown solutions, which, unlike those of violuric acid, are stable. In the presence of sodium acetate, it gives brown precipitates with Ni^{++} and Co^{++}, an orange precipitate with Ag^{+} and a greenish brown color with Cu^{++}.

Reduction of Alloxan-5,6-dioxime (VII).—One gram of alloxan-5,6-dioxime was suspended in 25 cc. of water and to this was added 10 cc. of 20% ammonium sulfide solution. The orange-colored mixture was gently heated until the reaction occurred and all went into solution. It was then boiled to remove the excess of ammonium sulfide, and finally filtered to separate the sulfur formed. The addition of dilute sulfuric acid to the filtrate gave 0.45 g. of 5,6-diaminouracil sulfate dihydrate,[12] which was recrystallized from hot water and analyzed.

Anal. Calcd. for $(C_4H_6O_2N_4)_2 \cdot H_2SO_4 \cdot 2H_2O$: C, 23.0; H, 4.3%. Found: C, 23.3; H, 5.1%.

Alloxan-6-oxime-5-phenylhydrazone (VIII).—The preparation of this product was similar to that of the dioxime given above. An 80% yield of brilliant yellow platelets, m. 268° (dec.) was obtained.

Anal. Calcd. for $C_{10}H_9O_4N_5$: C, 48.6; H, 3.6; N, 28.4%. Found: C, 48.2; H, 4.6; N, 28.8%.

Reduction of Isovioluric Acid. Isouramil (XI).—To 7.8 g. of isovioluric acid dihydrate suspended in 100 cc. of water was added 50 cc. of 20% ammonium sulfide. The reaction mixture was gently shaken. The reaction occurred readily with slight evolution of heat. The reaction mixture was then largely evaporated under diminished pressure to remove ammonia and hydrogen sulfide. The residue consisted of an aqueous suspension of isouramil and sulfur, which was acidified with acetic acid, and filtered. The solid was washed with water, alcohol and ether, and then extracted with carbon disulfide for one hour in the apparatus of Clarke and Kirner[13] to remove the sulfur. This treatment left 5.2 g. (91% of theory) of a flesh-colored powder which turned deep pink on standing.

Anal. Calcd. for $C_4H_5O_3N_3$: C, 33.6; H, 3.5; N, 29.4%. Found: C, 32.7; H, 3.8; N, 29.3%.

Isouramil does not melt below 290°. It is insoluble in hot water, alcohol or acetic acid. It dissolves in sodium hydroxide, ammonia and dilute hydrochloric acid. With barium hydroxide it gives a purple precipitate which turns white with excess of the reagent. An ammoniacal solution of isouramil gives a lilac precipitate with barium chloride and a deep blue color with ferric chloride. It reduces neutral or ammoniacal silver nitrate as well as Fehling's solution in the cold.

Hydrolysis of Isouramil to Dialuric Acid (XII).—A solution of one-half g. of isouramil in 10 cc. of dilute hydrochloric acid (1:1) was boiled for a few minutes and then cooled. A deposit of crystals weighing 0.25 g. was obtained, which melted at 214° (dec.) and gave a beautiful purple precipitate with barium hydroxide (which was not discharged by excess of the reagent). These properties characterized the product as dialuric acid.

Oxidation of Isouramil to Alloxan (III).—To 1.43 g. of isouramil was gradually added a mixture of 1.1 cc. of concentrated nitric acid and 1.1 cc. of water. The reaction proceeded with the vigorous evolution of gas. The mixture was finally warmed gently to complete the reaction and 5 cc. of glacial acetic acid added. On standing, 1.00 g. of large transparent crystals separated. These gave a white precipitate with barium hydroxide, and violuric acid with hydroxylamine by hydrochloride. These properties identified the product as alloxan hydrate.[14]

Acetylation of Isouramil.—One-half g. of isouramil was suspended in 10 cc. of acetic anhydride and the mixture boiled for half an hour. Complete solution did not occur during this time, but on adding boiling water, all the solid went into solution. On cooling, there was obtained 0.25 g. of a white powder, which darkened but did not melt below 275°. The product gave no color with ferric chloride and ammonia and dissolved in barium hydroxide to a colorless solution, indicating that the 5-hydroxyl group of isouramil had been masked. This was confirmed by the following analysis:

Anal. Calcd. for $C_6H_7O_4N_3$: C, 38.9; H, 3.8; N, 22.7%. Found: C, 39.0; H, 4.0; N, 23.1%.

When boiled in dilute hydrochloric acid for a few minutes the product was hydrolyzed to dialuric acid (barium hydroxide test).

Summary

1. The action of nitrous acid on isobarbituric acid yields isovioluric acid (alloxan-6-oxime), which is rearranged to violuric acid (alloxan-5-oxime) by means of acid, but otherwise behaves much like alloxan.

2. Reduction of isovioluric acid yields isouramil (5,6-dihydroxycytosine), which resembles dialuric acid in its chemical properties, and is converted into the latter by means of dilute acid.

[1] Baeyer, *Ann. Chem.*, **127**, 200 (1863); **130**, 140; **131**, 292 (1864).

[2] Ceresole, *Ber. chem. Ges.*, **16**, 1133 (1883).

[3] Behrend and Roosen, *Ann. Chem.*, **251**, 235 (1889).

[4] Biltz and Paetzold, *Ann. Chem.*, **433**, 64 (1923).

[5] Davidson and Baudisch, *J. Biol. Chem.*, **64**, 619 (1925).

[6] Hantzsch and Isherwood, *Ber. chem. Ges.*, **42**, 986 (1909); Hantzsch and Issaias, *Ibid.*, 1000.

[7] Traube, *Ber. chem. Ges.*, **33**, 1371 (1900); *Ann. Chem.*, **432**, 266 (1923).

[8] Biltz, Heyn and Bergius, *Ann. Chem.*, **413**, 68 (1916).

[9] Wheeler and Johnson, *J. Biol. Chem.*, **3**, 183 (1907).

[10] Gregory, *J. prak. Chem.*, [1], **32**, 278 (1844), showed that dialuric acid gave a white precipitate with barium ion, but that it was readily oxidized by air to alloxantine, which gave a purple precipitate. The role of air in the formation of a purple barium salt from dialuric acid was also appreciated by Liebig and Wöhler, *Ann. Chem.*, **26**, 279 (1838). For a modern interpretation of the color of barium alloxantinate, see Hantzsch, *Ber. chem. Ges.*, **54**, 1267 (1921) and Retinger, *J. Am. Chem. Soc.*, **39**, 1059 (1917).

[11] Davidson and Baudisch, loc. cit.

[12] Traube, loc. cit. Levene and Senior, *J. Biol. Chem.*, **25**, 607 (1916).

[13] Clarke and Kirner, *Organic Syntheses*, **2**, 49 (1922).

[14] Biltz, Heyn and Bergius, loc. cit. Ceresole, loc. cit.

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*CONCENTRATION OF THE H^2 ISOTOPE OF HYDROGEN BY THE FRACTIONAL ELECTROLYSIS OF WATER**

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If the reactions

$$X^+ + e = X$$
$$Y^+ + e = Y$$

are both possible cathodic reactions, the one having the lower cathodic potential will be favored to the practical exclusion of the other during an electrolysis under equilibrium conditions. Though the normal electrode potentials of the isotopes of all elements except hydrogen must be so nearly the same that no appreciable separation can be expected from any small differences, this may not be true in the case of the hydrogen isotopes because of the very large mass ratio.

In addition to a possible small difference in the normal electrode potentials of these two isotopes, the mechanism of the formation of hydrogen gas at the cathode involves the diffusion of the two species of ions through the cathode film and the subsequent union of the discharged ions to produce hydrogen molecules, in both of which processes (differences in "polari-

zation") there exists the possibility of a fractionation of the mixture, probably with a resulting enrichment of the residual water in respect to H^2, the species present at the smaller concentration. In that case it is obvious that a systematic fractionation by electrolysis should lead to two final fractions consisting, respectively, of (1) pure H^1 and (2) the equilibrium (i.e., electrolysis equilibrium) mixture of H^1 and H^2. It is impossible to predict the composition of this equilibrium mixture. While it might conceivably consist of almost pure H^2, it will probably be found to contain the H^2 isotope in a certain maximum concentration beyond which, practically speaking, it will not be possible to go.

On the basis of the above reasoning it appeared possible to concentrate the H^2 isotope of hydrogen by the electrolysis of water, and such an experiment was started at the Bureau of Standards on December 9, 1931. This experiment is still in progress and it is planned to compare the physical properties of the residual water with those of ordinary water and also to examine both the hydrogen and the oxygen of the residue, since some concentration of O^{18} as well as of H^2 appears possible.

Meantime, it seemed worthwhile to examine some of the residues obtained from the commercial electrolysis of water for the production of oxygen, although the conditions under which commercial cells are operated are rather unfavorable, owing to the continual additions of fresh water to the cells. Through the courtesy of the Southern Oxygen Company of Alexandria, Va., and later of the Ohio Chemical Company of New York, samples of the residual solutions from cells continuously operated for two and three years, respectively, were secured. The hydrogen and oxygen from this water have been examined at Columbia University.

The hydrogen was examined spectrographically as described by Urey, Brickwedde and Murphy.[1] No quantitative estimate of the abundance of H^2 can be made by this method, but the photographs show that there is a very definite increase in the abundance of H^2 relative to H^1 in these residual solutions.

The oxygen was combined with nitrogen to form nitric oxide and any change in the relative abundances of the oxygen isotopes was estimated from the relative intensities of the absorption bands of the molecules $O^{16}N^{14}$ and $O^{18}N^{14}$ using ordinary nitric oxide for comparison.[2] The oxygen analysis indicated a decrease of about 8 per cent in the concentration of O^{18} but the uncertainty in the estimation is about this large and therefore the change cannot be regarded as significant.

The above results are of great importance for we now know that there are large quantities of water in these electrolytic cells containing H^2 in relatively high concentrations and also there is available now a method for concentrating this isotope in large quantities.

In selecting from the determination in the literature, a value for the

relative abundances of H^1 and H^2 in natural terrestrial hydrogen, it is now necessary to know the past history of the hydrogen sample used by the observer and some of the discrepancies reported may be due to differences in the samples used.[3]

In addition to the electrolysis method for fractionating water, the distillation method could also be utilized by the unidirectional evaporation of a saturated brine at a sufficiently low temperature. While the theorectical efficiency of the process is less than that for the evaporation of liquid hydrogen itself, the experimental technique is much simpler and the process could be operated continuously with very little attention.

Various heterogeneous chemical reactions suggest themselves also as methods for securing fractionation of the two isotopes. Thus treatment of a large quantity of hydrochloric acid with iron or zinc should lead to a concentration of the H^2 isotope in the undecomposed residual acid.

* Publication approved by the Director of the Bureau of Standards of the U. S. Department of Commerce.

[1] Harold C. Urey, F. G. Brickwedde and G. M. Murphy, *Phys. Rev.*, **39**, 164 (1932); **39**, 536 (1932).

[2] See a forthcoming paper by G. M. Murphy and Harold C. Urey for experimental details.

[3] R. T. Birge and D. H. Menzel, *Phys. Rev.*, **37**, 1669 (1931); Walter Bleackney, *Phys. Rev.*, **39**, 536 (1932); Harold C. Urey, F. G. Brickwedde and G. M. Murphy, *Phys. Rev.*, **40**, 464 (1932); Kallman and Lazareff, *Naturwiss.*, **12**, 206 (1932).

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*THE ELECTRONIC STRUCTURE OF THE NORMAL NITROUS OXIDE MOLECULE**

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In a recent note[1] I applied a table of atomic radii for use in covalent molecules in predicting values of the moment of inertia of nitrous oxide, assuming various structures for the normal state. The values given are the following:

$:\ddot{N}::N::\ddot{O}:$	75.8	66.7	58.1×10^{-40} g. cm.^{2}
$:N:::N:\ddot{O}:$	75.3		
$:\ddot{N}:N:::O:$	75.2		

The three reasonable structures of the Lewis type lead to a moment of inertia of about 75×10^{-40} g. cm.^{2}. The structure corresponding to resonance between the first two Lewis structures gives a lower value, and that to resonance among all three a still lower value. It was pointed out in the note that the experimental value 59.4×10^{-40} g. cm.^{2} reported by Plyler and Barker agrees with the last of these to within about 2%, supporting the corresponding structure.

Professor R. M. Badger of this Laboratory observed that Plyler and Barker's data actually lead to 65.94×10^{-40} g. cm.^{2}, the lower value given by them being in error. The earlier conclusion regarding structure is consequently invalidated. The correct moment of inertia, however, agrees to within about 1% with the value 66.7×10^{-40} g. cm.^{2} predicted for the structure resonating between the Lewis structures $:\ddot{N}::N::\ddot{O}:$ and $:N:::N:\ddot{O}:$. The wave function for the normal nitrous oxide molecule is accordingly approximately represented by the sum of those for these two Lewis structures, with nearly the same coefficients, the contribution of the third Lewis structure given above being small. The nitrogen—nitrogen bond resonates between a double and a triple bond, approaching the latter in character, and the nitrogen—oxygen bond resonates between a single and a double bond, its properties being essentially those of a double bond. The structure might accordingly be written $N{\equiv}N{=}O$, even though the nitrogen atom cannot form more than four coexistent covalent bonds.

The choice of two of the three Lewis structures appears reasonable in the light of the rule, expressed by many authors, that stable electronic structures tend to leave atoms neutral or with small charges on assigning electrons to nuclei by splitting shared electron pairs. The central nitrogen atom in all three structures is N^+, this being required in order for it to form four covalent bonds. The first two structures, N^-N^+O and $N\,N^+O^-$, involve only single charges, and are essentially equivalent, contributing equally to the normal state, while the third, $N^{--}N^+O^+$, with a doubly-charged ion at one end, and two positive ions in juxtaposition, is less stable, and is not appreciably involved in the normal molecule.

* Contribution from Gates Chemical Laboratory, California Institute of Technology, No. 320.

[1] Linus Pauling, these PROCEEDINGS, **18**, 293 (1932).

FURTHER CONCERNING A PARASYMPATHETIC CENTER IN THE INTERBRAIN

VII. THE EFFECT OF INTRAVENTRICULARLY-INJECTED HISTAMINE

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Communicated May 28, 1932

In a series of papers published in these PROCEEDINGS a year ago,[1] it was pointed out:

(1) That the intraventricular injection of pituitrin, contrary to its known effects when given subcutaneously, caused a marked reaction characterized in susceptible subjects, by a bright flush, sweating, salivation, lachrymation, prolonged vomiting and fall in body temperature;

(2) That intraventricular pilocarpine in far smaller doses than were effective when given subcutaneously causes a highly similar reaction;

(3) That atropine serves to check these reactions;

(4) That they do not occur when the third ventricle is distended by marked hydrocephalus or when it is the seat of tumors; and

(5) That the reaction is annulled when the subject is under the influence of an hypnotic drug which presumably exercises its effects directly upon the hypothalamic centers.

From these observations the inference was drawn that pituitrin and pilocarpine given intraventricularly acted directly upon a higher vegetative center in the interbrain which appeared to be essentially parasympathetic in nature.

That the interpretation of these reactions ran counter to certain recognized pharmacological tenets was fully realized. One of them concerns the principle that a drug wherever introduced into the body necessarily produces the same effect, differences in rate of absorption and individual susceptibility, of course, being taken into consideration. Another generally accepted principle, which the observations tended to discredit, is that parasympathetic stimulants like pilocarpine acted on the nerve terminals rather than centrally.

Objections on these scores were fully anticipated but a serious criticism I was unprepared to meet was promptly received from my friend, Professor J. J. Abel, who struck at the very root of the matter. He expressed the conviction that the flushing effects which had been observed must certainly be due to impurities in the extract—to *histamine* in all probability. It was obvious that should this prove to be true, the entire thesis which had been primarily built up on the intraventricular reaction to pituitrin would fall to the ground.

To meet this criticism, two courses were open: (1) to analyze commercial

pituitrin for the presence of histamine or other flushing bodies; and (2) to see whether intraventricular histamine gave effects comparable to those produced by intraventricular pituitrin. Professor Abel generously volunteered to investigate the first point and, after a year's interval, I have finally got round to putting the second to clinical test.

Though the manufacturers of the posterior lobe extract which goes by the name of "Pituitrin-S" felt certain that their product was histamine-free, Dr. Kamm, of Parke, Davis and Company, kindly put at Dr. Abel's disposal a liter of the substance for his personal analysis. And I think I am correct in stating at this time that it proves to contain no recoverable histamine though he remains convinced that it must hold impurities of some other sort, which give the flushing effects.

From time to time during the year, Dr. Abel has forwarded from Baltimore a number of extractives from pituitrin containing possible impurities as well as samples of highly concentrated active principle which have been tested by introducing them into the ventricles of monkeys. These tests which have been carried out in the Laboratory of Surgical Research at the Harvard Medical School by Dr. Richard Light with the assistance of Dr. Stanley Bysshe, are not only difficult to make but difficult to interpret as the reactions of animals to intraventricular injections correspond only in a general way to those observed in man, as will be gathered from their report. In regard to histamine, it may be said that five times the dose of this substance needed to produce flushing effects when given intravenously has no appreciable pharmacological effect when injected into the monkey's ventricle. And the same is true of acetylcholine.

So far as histamine is concerned, I fully expected that its intraventricular injection in man would give results comparable to those produced by intraventricular pilocarpine and pituitrin: namely, flushing, sweating, vomiting and so on. As a matter of fact, much to my surprise, the customary dose of histamine employed clinically for testing the acid-producing capacity of the gastric mucosa (viz., 1 to 2 mgm. of histamine dihydrochloride), when injected into the human ventricle, gives no flushing effects. This has now been tried on three patients, all of them adults satisfactorily convalescing from operations for typical chromophobe adenomas large enough to compress the optic chiasm and destroy the posterior lobe but wholly confined within an intact diaphragma so that tuber and third ventricle were intact. The results are given in the following abbreviated protocols:

Effect of Intraventricular Histamine

Case 1.—A woman 39 years of age with no constitutional evidences of dyspituitarism and a normal basal metabolic rate, convalescent from her operation, after the usual preliminary observations was subjected to the following comparative tests: (1) with *intraventricular pituitrin;* (2) with *intraventricular histamine;* (3) with *intramuscular histamine.*

First test (January 19, 1932).—One cubic centimeter of *surgical pituitrin* was injected *in the lateral ventricle*. A characteristic though somewhat delayed reaction appeared in 36 minutes—slight flush with sweating followed by prolonged nausea, vomiting and evacuation of bowel. The vagal reaction was so prolonged it was ultimately checked by morphine and atropine. There was a slight fall in temperature. No change in basal metabolic rate.

Second test (January 21, 1932).—Two milligrams of *histamine* were injected *in the lateral ventricle*. Fairly prompt pallor of face despite subjective feeling of warmth and sense of fullness in head. Slightly increased moisture of skin. Belching and two attacks of vomiting, one after 30 minutes and the second after an hour. The last specimen showed 35° free acid and 44° total acid.

Third test (January 23, 1932).—Rehfuss tube with three pre-injection observations with 20-minute intervals on gastric secretion. *Intramuscular injection of 1 mgm. histamine*. Within two minutes, a marked generalized flush of face and neck including the skin over the bone flap. Sense of fullness in head. No sweating or nausea. Flush beginning to fade after 20 minutes and gone at the expiration of an hour. The three pre-injection tests of gastric secretion averaged 66 cc. in amount with free acid 12° and total acid 18°. The three post-injection tests averaged 42 cc. in amount with 46° free acid and 45° total acid.

The tests, in short, showed a typical flushing reaction to intramuscular histamine which included the anesthetic skin over the bone flap. Hence, the dilator effect must be blood-borne rather than through vasodilator fibres accompanying the sensory nerves, as appears to be the case with intraventricular pituitrin and pilocarpine. The typical increase of gastric acidity without vomiting followed the intramuscular injection and the single test of the vomitus after intraventricular histamine suggested that in the absence of flushing the same effect had taken place. To make this point more certain, in the following case a Rehfuss tube was in place during the intraventricular tests.

Case 2.—A man 36 years of age, six days convalescent from his operation and showing no constitutional evidences of dyspituitarism apart from a low blood-pressure and subnormal (−26) basal metabolic rate. Comparative tests were made (1) with *intraventricular pituitrin*, (2) with *intramuscular histamine* and (3) with *intraventricular histamine*.

First test (January 29, 1932).—The usual preliminaries showed a rectal temperature 98.4°, pulse 50, blood-pressure 82/52. At 10.40 A. M., *one cubic centimeter of surgical pituitrin was introduced into the ventricle*. Within one minute the patient complained of a sense of gastric uneasiness. Within two minutes his face flushed, the bone flap being spared, and he vomited (0 free acid; total acidity, 24°). There was a temporary rise in blood-pressure to 100/70 with increase in pulse rate to 76. The flush increased and mild sweating first appeared after 12 minutes. Second attack of vomiting after 50 minutes (0 free acid; total acidity 14°, guaiac +). Sweating and flushing practically disappeared after 1 hour 20 minutes, but nausea and belching persisted with occasional vomiting of small amounts (24 cc., containing 0 free acid; total acidity 7°, guaiac +) until 3 P. M. when the reaction was checked by morphine and atropine. There was a fall in rectal temperature of one degree only, to 97.4°, with a subsequent thermic rebound to 101.4° at 6 P. M.

Second test (February 2, 1932).—The usual preliminaries with added introduction of a Rehfuss tube. Three 20-minute specimens of gastric juice showed an average of free

acid 26° and total acid 36°. At 9.45 A. M., *0.75 mgm. of histamine* was injected into the deltoid muscle. Within one minute the patient flushed brightly, the skin over the bone flap sharing equally with the rest of the face. There was complaint of slight headache—"like smelling amyl nitrite"—but no sweating or nausea. The flush gradually faded and was no longer appreciable after 30 minutes. Meanwhile, there had been no discernible change in pulse, respiration, temperature or blood-pressure. The post-injection gastric specimens showed after 20 minutes (35 cc. containing 58° free acid, 68° total acid), after 40 minutes (25 cc. containing 74° free and 85° total acids), and after 60 minutes (22 cc. containing 70° free and 93° total acids; no guaiac).

Third test (February 3, 1932).—The usual preliminaries. All but one of the pre-injection amounts of gastric juice withdrawn from the Rehfuss tube were too small accurately to measure. This single specimen showed 2° free and 25° total acid.

At 10.33 A. M., *1.5 mgm. histamine* (double the dose of the second test) was *injected into the ventricle.* Almost immediately the patient complained of headache but there was no observable reaction other than a slight pallor of the face and momentary subjective uneasiness of the stomach. At 11 A. M., to check his headache he was given 0.5 mgm. of morphine. The post-injection gastric specimens showed: after 20 minutes (19° free and 40° total); after 40 minutes (50° free and 71° total); after 60 minutes (80° free and 90° total); after 80 minutes (80° free and 92° total); after 100 minutes (76° free and 83° total); after 120 minutes (46° free and 65° total acids). At no time were amounts of more than 10 cc. of gastric juice secured; none of the specimens showed a positive guaiac test.

From the foregoing tests, it would appear that the intraventricular injection of histamine causes facial pallor rather than flushing even in doses twice that which evokes an almost immediate and prolonged flush when injected in the muscle. Hence, the striking flushing effects produced by intraventricular pituitrin can scarcely be ascribed to the absorption into the blood-stream of associated histamine, whose molecule, if I am correctly informed, is smaller than that of pituitrin and should be absorbed more rapidly.

In a recent report by Weiss, Robb and Ellis[2] on the systemic effects of histamine, it has been shown that the responses to histamine in man and animals differ widely. Of particular interest is their demonstration that the transient flushing effects are particularly marked in the brain, as shown not only by increased pulsatile expansion of the cerebrospinal fluid but also by direct observation during the course of a cerebral operation. Moreover, there is customarily an increase in basal metabolic rate and a rise in blood-pressure rather than the fall which occurs in anaesthetized animals.

In the following case, the order of the injections was altered to leave the more upsetting reaction from intraventricular pituitrin to the last.

Case 3.—A man 46 years of age, three weeks convalescent from an operation which left him with some evidences of pituitary deficiency shown particularly by his low basal metabolic rate. Tests were made: (1) with *intramuscular histamine;* (2) with *intraventricular histamine;* (3) with *intraventricular pituitrin.*

For convenience of comparison, the effects of the histamine injections are put in tabular form and made to synchronize as though both were given at 10 A. M. As a

matter of fact, the intramuscular injection was given at 10.01 A. M. and the intraventricular injection at 9.31 on the following day. On each occasion preliminary observations were made with the patient fasting, beginning with an estimation of the basal metabolic rate. The Rehfuss tube was then swallowed and gastric contents removed every twenty minutes, the last just before the injection. Pulse, respiration and blood-pressure were plotted at frequent intervals and a constant chart of rectal temperature was electrically recorded on a drum. The patient was quiet and coöperative throughout.

INTRAMUSCULAR HISTAMINE (MAY 3, 1932)	(TIME)	INTRAVENTRICULAR HISTAMINE (MAY 4, 1932)
Basal metabolic rate −32. Gastric juice, two preliminary observations; fluid colorless and watery (specimens averaged 15 cc. with 47° free acid, 59° total acid). Pulse 80; respiration 20; blood-pressure 120/80. Well covered; comfortable; rectal temperature 98.5°, room temp. 76°.	8.30 A.M.	Basal metabolic rate −32. Gastric juice, colorless and watery (two specimens averaged 25 cc. with 48.5° free acid, 60° total acid). Pulse 80; respiration 20; blood-pressure 100/80. Comfortable. Rectal temperature 98.6°; room temperature 82°.
Injection in deltoid muscle 1 mgm. histamine dihydrochloride. Prompt feeling of warmth followed by bright flush, including bone flap. Skin dry. Transient elevation of pulse to 110 with fall in blood-pressure to 105/70.	10.00	*Injection to ventricle 1 mgm. histamine.* Almost immediate sense of warmth with fleeting suggestion of flush and slight moisture on chin. No change in pulse, respiration or blood-pressure.
Condition stationary. Flush persists; no sweating. Gastric contents (amount 18 cc.: 87° free acid, 96° total acid; 0 guaiac).	10.20	Comfortable; composed. Slight headache. Slight increase cutaneous moisture of face persists. Gastric juice (13 cc.: 71° free and 80° total acid).
	10.30	Headache more severe; slight nausea.
Flush beginning to fade. Pulse 90, respiration 25. Blood-pressure stationary at 110/80. Second specimen gastric contents (12 cc., light brown color: 110° free acid, 115° total acid; faintly positive guaiac).	10.40	Pulse, respiration, blood-pressure remain unaltered. Gastric juice (14 cc.: 10° free and 20° total acid; faint guaiac).
Flush still present. A slight rise in rectal temperature to 98.9°. Third gastric specimen (12 cc., medium brown color: 103° free acid, 106° total acid; positive guaiac).	11.00	Headache much less. Third gastric specimen (13 cc., clear and watery: 10° free and 21° total acid; positive guaiac).
Slight flush still present. Rise of 1° rectal temperature. Fourth gastric specimen (10 cc., coffee-brown color: 84° free acid, 99° total acid; strongly positive guaiac). Rehfuss tube withdrawn.	11.20	Complains of feeling cold though body warm to touch. Rectal temperature 97.9°, a fall of 0.7°. Fourth gastric specimen (10 cc. colorless fluid: 10° free, 19° total acid; guaiac + +).
Basal metabolic rate −13, a rise of 19 points. Observations discontinued.	11.40	Fifth gastric specimen (only 3-4 cc. colorless fluid obtainable: (guaiac + +). Rehfuss tube withdrawn.
	12.00	Basal metabolic rate −19, a rise of 13 points. Observations discontinued.

The foregoing comparative tests with histamine show, in accordance with what is well known, that intramuscular histamine in 1 mgm. doses gives a bright, prolonged flush with a sense of fullness in the head, a temporary rise in blood-pressure and pulse rate, and increased gastric acidity in the absence of any notable increase of secretion. On the other hand, intraventricular histamine in equal amount gave a trifling momentary increase of color scarcely to be called a flush, caused headache, some nausea and a possible temporary increase in gastric acidity, quickly followed by diminution below the pre-injection percentages.

Test 3.—To make certain that the patient was normally susceptible to *intraventricular pituitrin*, on May 7 he was given the customary 1 cc. by this route. After an hour's interval (during which he showed a rapid increase in gastric acidity which rose to 100° free and 106° total acid with positive guaiac test), a severe reaction set in culminating in a brilliant flush sparing the bone flap, drenching perspiration, vomiting and a fall in rectal temperature of 1°, to 97.8°.

The reaction lasted for fully two hours during which time the brilliant flush and accompanying sweat would fade and reappear in waves averaging from ten to fifteen minutes in duration. The over-severe reaction was finally subdued by an injection of morphine and atropine. There was a subsequent thermic rebound four hours later to 101.4°, a rise of four degrees.

These three observations on the pharmacodynamic effect of intraventricular histamine (in patients shown to be highly susceptible to intraventricular pituitrin) in view of the negligible response would appear to exclude a histamine impurity as the cause of the pituitrin reaction. Whether pituitrin contains acetylcholine in amounts sufficient to account for its flushing effect when injected into the ventricle seems highly improbable. While animals are highly susceptible to the effects of acetylcholine, man appears to be highly resistant. Administered intravenously or subcutaneously in doses vastly greater than could possibly be carried as an impurity in a cubic centimeter of pituitrin, its parasympathetic effects prove to be exceedingly transient. It therefore has seemed unnecessary to subject patients to the test of its intraventricular effects.

[1] *Proc. Nat. Acad. Sci.*, **17**, 163–180, 239–264 (1931).

[2] Weiss, S., Robb, G. P., and Ellis, L. B., "The Systemic Effects of Histamine in Man," *Arch. Int. Med.*, **49**, 360–396 (1932).

VIII. THE COMPARATIVE EFFECTS ON GASTRIC MOTILITY OF INTRAMUSCULAR AND INTRAVENTRICULAR PITUITRIN, PILOCARPINE AND HISTAMINE

While the tests described in the preceding section were being conducted, certain studies on gastric motility, as affected by the intramuscular or intraventricular injection of pituitrin, pilocarpine and histamine, have been made with the friendly coöperation of Dr. M. C. Sosman, roentgenologist to the Brigham Hospital, and his assistant, Dr. H. F. Hare.

As the results of these tests are pertinent to the mooted point regarding the essential difference of the reaction to centrally and peripherally administered pituitrin, they may be put on record at this time though deserving of further expansion.

The observations which have been made on a series of eleven patients, six of them after operations for chromophobe adenomas, have been almost wholly restricted to the single point of gastrointestinal motility. Having been necessarily conducted in a dark room, the usual detailed consecutive records of pulse, temperature, blood-pressure, color change and so on have been impracticable and made only at irregular times.

Pituitrin Effects.—It has long been known that in addition to its antidiuretic effect, intramuscular or intravenous pituitrin causes cutaneous pallor together with a slight rise in blood-pressure and pulse rate suggesting a sympathico-adrenal response. Intraventricular pituitrin, on the other hand, produces a cutaneous flush, sweating, salivation and vomiting, which were looked upon as of central, parasympathetic origin. It seemed highly improbable that the active principle of an organ of internal secretion should have a contrary action if introduced in different regions of the body, though this was the only interpretation of these effects that could be readily offered. What effect on gastric motility intramuscular pituitrin would have was unknown though the prolonged retching and vomiting that followed its intraventricular injection suggested a central stimulation of the vagus in which, curiously enough, the fibres to the heart did not participate. We were wholly unprepared for the gastric response which actually occurs.

Case 1.—A patient satisfactorily convalescing from an operation for the removal of a parietal glioma was given an opaque meal (90 gm. barium sulphate in 200 cc. malted milk). Vigorous and unusually active peristalsis of three-wave type started immediately. On the third wave emptying began and the barium column shot into duodenum and jejunum. Six or eight waves were followed through from cardia to pylorus. A cc. of *surgical pituitrin* was then injected into the deltoid muscle and within 30 seconds there was *complete cessation of all peristaltic movement* in the stomach and shortly after in the intestine. After an interval of 20 minutes, sluggish movements were resumed, first in the intestine, and ten minutes later normally active gastric peristalsis was fully restored.

Precisely this same effect was observed in the case of John S., whose comparative records of intraventricular pilocarpine and pituitrin were given in the second instalment in this series (cf. pp. 172–173). He had remained well and at work for nearly a year and had recently reëntered the hospital because of a recurrence of his tumor symptoms. A secondary operation had been performed with such satisfactory results he in gratitude willingly submitted to another series of tests, as follows:

Case 2 (John S.).—After a barium meal, fluoroscopic studies were made to determine:

(1) the effect of *intramuscular pituitrin;* (2) of *intraventricular pituitrin;* (3) of *intramuscular pilocarpine;* and (4) of *intraventricular pilocarpine.*

Test 1 (March 24).—After ingestion of the barium, active peristalsis started immediately, deep vigorous waves progressing from the cardiac third to pylorus, the stomach emptying on the first wave. A succession of waves were timed through at an average of 37 seconds.

Injection.—A cc. of *surgical pituitrin* was then injected into the deltoid muscle and within 30 seconds all gastric movement had ceased, the jejunal peristalsis continuing for a few seconds longer. After a five-minute interval, jejunal peristalsis was resumed but 20 minutes elapsed before the slightest movement in the stomach was discernible. By this time the head of the barium calcium in the intestine had reached the caecum. Shortly after, he had a loose stool.

Test 2 (March 30).—While recumbent, at 10.10 A. M., a lumbar puncture needle was inserted in the ventricle and a few drops of fluid allowed to escape. At 10.12, the barium meal was given and vigorous peristalsis of three-wave type immediately started. Several waves were timed through at an average of 35 seconds.

Injection.—At 10.20, *surgical pituitrin* (1 cm.) *was injected into the ventricle* and the needle withdrawn. Peristaltic waves remained active and vigorous and so rapid they were difficult to time. There was such rapid emptying that further barium was given at 10.34, some of it being regurgitated. At 10.48, no visible trace of barium remained in the stomach, most of it being in the lower ileum. At 11.35, the head of the barium column was in the hepatic flexure; no stool 'till following morning.

The general reaction (as the year before) to this 1 cc. of intraventricular pituitrin was slight, there being a delayed flush, a moderate sweat and an attack of vomiting at 12.30 P. M. (250 cc. with free acid 6° and total acid 36°). There was a fall in rectal temperature from 98.6° to 97.4° at 1.45, followed by a thermic rebound to 101.6° at 9 P. M.[3]

Pilocarpine Effects.—On this same patient, for comparison with the foregoing, the following tests with pilocarpine were made.

Test 3 (April 5).—Again after the barium meal vigorous, deeply-cut, three-wave peristalsis began immediately with rapid emptying: waves timed at 40 seconds.

Injection.—At 10.11 A. M., 12 mgm. of *pilocarpine were injected into the deltoid muscle.* At 10.16, subjective feeling of warmth with sweating; also a definite diminution in activity of peristalis though movement was not so completely inhibited as with subcutaneous pituitrin. At 10.26, duodenal cap emptied for the first time after the injection. At 10.31, vigorous peristaltic rushes were resumed in the ileum though activity of gastric movement was still retarded and remained so until 10.44 when the observation was concluded.

Test 4 (April 7).—Again, 90 gms. barium in malted milk followed by immediate active peristalsis of three-wave type with rapid emptying. "Wave-time" averaged 40 seconds.

Injection at 11.14 A. M. of 2.5 mgm. *pilocarpine intraventricularly.* In 4 minutes, a subjective sensation of warmth. This was accompanied by pylorospasm and antiperistaltic waves leading to three attacks of vomiting at 11.18, 11.19 and at 11.21. Each attack was accompanied by vigorous antiperistalsis. Under the light, the patient was seen to be having a marked parasympathetic effect with bright flush, profuse sweat and salivation such as he had shown before. On the cessation of vomiting, the

[3] In the second paper in the series, in which the general reaction was described, stress was laid on the primary drop in temperature, and the fact that a thermic rebound is apt to take place was insufficiently emphasized.

tone of the stomach remained markedly increased with prominent and apparently swollen rugae.

At 11.26, 0.5 mgm. of atropine was given to check the explosive reaction. It was impossible subsequently to time the waves with any accuracy. The peristalsis in the small bowel meanwhile had continued and by 11.29 the barium column had reached the ileum.

By 11.31, all visible forward movement in stomach and intestine had ceased. Strong deep reverse peristaltic waves associated with spasm of the lower two-thirds of the stomach and crowding the barium into the fundus and through the cardia into the oesophagus subsequently occurred at irregular intervals, the patient being conscious of each of them even before the fluoroscopist could catch them. Some of these anti-peristaltic waves were accompanied by vomiting.

Not until 11.55 did the stomach begin to relax and show an occasional peristaltic wave in the normal direction. Fluoroscopy was then temporarily concluded. One more attack of vomiting occurred at 12.05 P. M. At 1.25 P. M., he was again fluoroscoped and the stomach found to be emptying with the head of the barium column in the caecum.

The general features of the reaction were precisely similar to that previously reported (cf. p. 172). At 1.15 P. M., the rectal temperature had dropped to 96° and from this low level there was a gradual rise to normal at 2.15 P. M. with a thermic rebound reaching 104° at 6 P. M. followed by slow subsidence to normal.

Tests for acid in the vomited fluids indicated that the increased tonus was unassociated with secretory activity as shown in the following table:

STUDIES OF VOMITUS

TIME OBTAINED	AMOUNT	CHARACTER	ACIDS FATTY	ACIDS TOTAL	GUAIAC
11.18	125 cc.	Greyish fluid	48	60	0
11.21	35 cc.	Reddish brown	20	43	++
11.34	40 cc.	Reddish brown	12	31	++
12.05	15 cc.	Brownish	18	41	+++

So far, it would appear: (1) that neither subcutaneous nor intraventricular pilocarpine or pituitrin increase gastric acidity as does histamine; (2) that intramuscular pituitrin and, to a less extent, pilocarpine inhibit gastric peristalsis whereas histamine does not notably affect it; and (3) that intraventricular pilocarpine and pituitrin increase motility to the point of pylorospasm with retrograde peristalsis and vomiting.

At about this time, a chance observation had been made on a patient with an unverified tumor of the third ventricle associated with a gastric ulcer who had returned for examination. Gastro-intestinal studies under a barium meal showed the ulcer to be healed. The stomach exhibited normal peristalsis of three-wave type, the wave-time averaging 65 seconds. He was given an *intramuscular injection of 12 mgm. pilocarpine* which in six minutes provoked the customary constitutional reaction with sweating and salivation and so on. No change, however, was apparent in gastric motility until 15 minutes had elapsed when the peristalsis became more vigorous, a 52-second wave being timed, 4 minutes later a 45-second wave, in another minute a 47-second wave and 4 minutes later a 46-second wave.

Knowing how prompt is the effect on the stomach of even small doses (2 mgm.) of intraventricular pilocarpine, it was thought that this delayed acceleration of peristalsis might be ascribable to the retarded action of the circulating drug on the parasympathetic or vagal center in the brain. The same delayed effect was also observed in another patient on whom the following series of tests were made:

Case 3.—The patient was a young man previously under observation with choked discs due to an unlocalizable tumor associated with a duodenal ulcer. A subtemporal decompression had been performed with subsidence of his choked discs and headaches together with apparent healing of the ulcer. The tests were planned to determine with the aid of a Rehfuss tube the comparative effects on gastric secretion and motility of *intramuscular pilocarpine, histamine and adrenalin.*

First test (April 5, 1932).—The pre-injection specimens of gastric juice averaged 77 cc. in amount with 17° free acid and 26° total acid.

8.02 A. M. *Injection, intramuscular, of 12 mgm. pilocarpine.* Within five minutes there was flushing, generalized profuse sweating, lachrymation and salivation. There was a temporary increase in pulse rate from 68 to 90, and the blood-pressure fell for 20 minutes from 128/80 to 118/70. No change in respiration or rectal temperature. The reaction persisted for an hour and by 9.20 was practically over.

At 8.25, just 25 minutes after the injection, he had some belching and complained of a bellyache which he said was precisely like his old familiar "hunger pains." This continued intermittently with some nausea and continued belching until finally at 8.55 it culminated in an attack of unproductive retching. Some nausea persisted for ten minutes and then passed off.

The acid content of the aspirated post-injection specimens of gastric juice appears in the following table:

TEST 1, CASE 3, ASPIRATED SPECIMENS OF GASTRIC JUICE

SPECIMEN	TIME	AMOUNT	ACIDS FREE	ACIDS TOTAL
1	8.20	170 cc.	10	20
2	8.40	170 cc.	0	12
3	9.00	45 cc.	0	4
4	9.20	5 cc.	6 (?)	12 (?)

In correspondence with the observation of others, therefore, intramuscular pilocarpine in this case diminished rather than increased the gastric acidity, acting, in other words, unlike histamine. The reaction was accompanied, however, by a delayed (23 min.) disturbance apparently affecting gastric motility. More precisely to determine the effect of the injection on motility was the purpose of the next test.

Second test (April 6, 1932).—After a barium meal peristalsis again of the three-wave type promptly set in with early emptying. Three waves were timed through averaging 53 seconds.

Injection at 10.41 A. M. of *12 mgm. pilocarpine* into deltoid muscle. Same constitutional reaction as with test of preceding day. There was no appreciable change in peristalsis—certainly no increase in activity, the next few waves being actually timed at 10 seconds slower than before. For the next 20 minutes peristalsis of original type continued unaltered.

At 11.03 A. M., 21 minutes after the injection, he began to complain, as on the preceding day, of "hunger ache." A peristaltic wave had just been timed at 61 seconds. At 11.05, a vigorous wave was timed at 50 seconds; at 11.06, at 46 seconds; at 11.07, at 48 seconds; at 11.13, at 47 seconds. Shortly after this his "ache" wore away and at 11.36 and 11.37 waves were again timed at *circa* 54 seconds. The observation at this point was discontinued.

This test, therefore, tended to confirm the suggestion of a delayed effect of intramuscular pilocarpine in accelerating peristalsis, possibly from a tardy action on the vagal center by the circulating drug. Comparative tests with intramuscular histamine were also on two occasions made on this same patient. They showed the usual increase in acidity and possibly a slight acceleration of motility with temporary shortening of the wave-time and a tendency to pylorospasm, but nothing striking.

In view of the suggestion that intramuscular pituitrin acted as a sympathico-adrenal stimulant, it remained to be seen whether adrenalin would have a corresponding effect on gastric motility. The test was made on the same patient, Case 3, as follows:

Third test (April 14).—Usual preliminaries with barium meal. Immediate inauguration of active peristalsis of three-wave type, the average wave-time being 49 seconds.

10.51 A. M. *Injection 1 mgm. adrenalin (1 cc. solution) intramuscularly.*—Usual prompt adrenalin reaction (rise in blood-pressure and slight increase in pulse rate) with prompt inhibition of peristaltic activity. The first post-injection wave started but stopped in course. It was followed by a few shallow incomplete waves and in 4 minutes there was complete cessation of movement both in stomach and intestine. This lasted for 20 minutes when gastric peristalsis again began to occur. The first wave capable of being followed through was caught at 11.33 A. M. and timed at 55 seconds. Normal peristalsis before this time had been resumed in the intestine.

Conclusions.—If these observations are correctly interpreted, pituitrin given intramuscularly has an effect contrary to that which occurs when injected into the cerebral ventricle. The systemic effects of the former method of administration are closely similar to those produced by adrenalin and suggest stimulation (probably peripheral) of the thoraco-lumbar sympathetic apparatus. The stimulatory effects of intraventricular pituitrin, on the other hand, are essentially parasympathetic in character resembling those of pilocarpine in that they produce flushing and sweating together with a hypervagotonic effect on the stomach leading to pylorospasm, retrograde peristalsis and vomiting, due apparently to efferent impulses of central origin.

ORDERS FOR WHICH THERE EXIST EXACTLY FOUR OR FIVE GROUPS

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We shall first suppose that there are exactly four groups of order $g = p_1p_2 \ldots p_\lambda$, where $p_1, p_2, \ldots p_\lambda$ are distinct prime numbers. If a prime factor of g which is congruent to unity with respect to another prime factor of g is thus congruent with respect to exactly two such factors then none of the prime factors of g except the first can be thus congruent with respect to a prime factor of g. As there are exactly four groups whose common order is the product of these three prime factors every group of order g is the direct product of one of these four groups and a cyclic group which may reduce to the identity. Each of the four former groups gives rise to a cyclic quotient group with respect to the invariant subgroup whose order is the first of the three given prime numbers.

When g involves four prime factors such that each of two of them is congruent to unity with respect to one and only one of the remaining two, respectively, but not with respect to another prime factor of g, and no prime factor of g besides the two given ones is congruent to unity with respect to another prime factor of g and the larger of these two is not thus congruent with respect to the smaller, then each of the four possible groups of order g is the direct product of three groups. The third of these groups is always cyclic and may reduce to the identity while the order of each of the other two is the product of two distinct prime numbers. In each of the two given cases when there are four groups of order g this order is evidently odd and there is no upper limit to the number of its prime factors. In the remaining case this order must always be even and involve three and only three prime factors since g must then involve two odd prime numbers such that the larger is not congruent to unity with respect to the smaller and the cyclic group whose order is the product of these two primes is extended by an operator of order 2.

If there are exactly five groups of order g then the largest prime factor of g which is congruent to unity with respect to another such factor p can obviously not be thus congruent with respect to two such factors. Hence it results that either p is congruent to unity with respect to another prime factor of g or one of these other factors is thus congruent with respect to p. In the latter case there are exactly five such groups of order g if and only if $p = 3$ and none of the prime factors of g except the two given ones is congruent to unity with respect to such a factor and these two are thus congruent only with respect to 3. It remains to consider

the case when p is congruent to unity with respect to another prime factor of g and hence there are exactly three groups whose common order is equal to these three prime factors. Therefore g must involve at least four prime factors. When either of the last two of the given three prime factors (but only one of them) is congruent to unity with respect to this fourth prime factor of g there are obviously five groups of order g and only then.

Hence, there are also three cases in which there are exactly five groups of order g when g is not divisible by the square of a prime number. In the last two of these cases each of the possible groups is the direct product of a group whose order involves four prime factors and a cyclic group which may reduce to the identity. In the first case each of the possible groups is the direct product of a group whose order involves three distinct prime factors and a cyclic group which may reduce to the identity. In all of these cases the orders of all the possible groups are odd. That is, *if there are five and only five groups of a given order which is not divisible by the square of a prime number then this order must be odd.* In what follows g will not be restricted to a number whose order is not divisible by the square of a prime number.

A necessary and sufficient condition that there are exactly four groups of order g when $g = p_1^2 p_2^2 p_3 \ldots p_\lambda$ is that all the groups of this order are abelian. That is, no $p_i^{\alpha i} - 1$, $i = 1, 2, \ldots \lambda$ is divisible by one of the primes $p_1, p_2, \ldots p_\lambda$.[1] The only form of g, when there are exactly four groups of order g, that remains to be considered is $p_1^2 p_2 \ldots p_\lambda$. If one and only one of the prime numbers $p_2 \ldots p_\lambda$ is congruent to unity with respect to p_1, but not with respect to p_1^2 nor with respect to another of these primes, while none of the other prime factors of g is congruent to unity with respect to such a prime factor or divides $p_1 + 1$, then each of the possible four groups of order g is the direct product of a group of order $p_1^2 q$, where $q - 1$ is divisible by p_1 but not by p_1^2, and a cyclic group which may reduce to the identity. The fact that there cannot be more than four groups of order g when these conditions are satisfied results from the proof that such a group contains exactly $p_1^2 q$ operators whose orders divide this number and that it could not involve p_1^2 subgroups of order q. Hence it must involve a cyclic invariant subgroup of order $p_1 q$ since it cannot contain exactly p_1 subgroups of order q.

If one and only one of the prime numbers $p_1 \ldots p_\lambda$ is congruent to unity with respect to another one of them and this is thus congruent with respect to only one of them while none of these numbers divides $p_1^2 - 1$ nor is any of these primes congruent to unity with respect to p_1, then each of the four groups of order g is the direct product of a group of order p_1^2, a group whose order is the product of the two given prime numbers, and a cyclic group which may reduce to the identity. Hence it results that *there are six cases when there are exactly four groups of order g.*

In four of these cases g must be odd, in one case it must be even, while in the remaining case it may be either odd or even.

When there are exactly five groups of order g this order may be of the form $p_1^3 p_2 \ldots p_\lambda$ provided no one of these prime numbers is congruent to unity with respect to another one of them and none of the orders of the groups of isomorphisms of the various groups of order p_1^3 is divisible by one of the prime numbers $p_2 \ldots p_\lambda$. Each of these five groups is the direct product of a group of order p_1^3 and a cyclic group, which may reduce to the identity. As there is obviously no order of the form $p_1^2 p_2^2 p_3 \ldots p_\lambda$ for which there are exactly five groups it remains only to consider the orders which are of the form $p_1^2 p_2 \ldots p_\lambda$, for which exactly five groups exist.

Suppose that g is of this form and that one and only one of the prime factors of g is congruent to unity with respect to another such factor and this is thus congruent with respect to p_1^2 but not with respect to any other prime factor of g nor does any such prime factor divide $p_1^2 - 1$. It is easy to prove that every group of order g must then involve exactly $p_1^2 q$ operators whose orders divide this number, where $q - 1$ is divisible by p_1^2. Such a group can therefore not involve as many as p_1^2 subgroups of order q and hence it involves only one such subgroup. It results that every such group is the direct product of one of the five possible groups of order $p_1^2 q$ and a cyclic group which may reduce to the identity. The smallest value of g for which exactly five such groups exist is 20. There are obviously also exactly five groups of order $2p_1^2$, p_1 being any prime number.

When $p_1 - 1$ is divisible by one of the prime numbers $p_2 \ldots p_\lambda$ there are always more than five groups of order g except when this prime is 2 and g reduces to $2p_1^2$. Hence we may assume in what follows that p_1 is incongruent to unity with respect to each of the prime factors of g and that none of these factors is congruent to unity with respect to p_1 except when $g = 12$. If $p_1 + 1$ is divisible by two and only two of the distinct prime factors of g but no such factor is congruent to unity with respect to another then each of the possible groups of order g contains an invariant subgroup of order p_1^2 and there are five groups of order g. This is also the case when $p_1 + 1$ is divisible by one and only one p of these prime factors and $p - 1$ is divisible by one and only one such factor while none of the other prime factors of g is congruent to unity with respect to such factors. The existence of exactly five groups in these cases results almost directly from the following elementary theorem: *If any group involves a cyclic Sylow subgroup of order p^m then all of its subgroups of order p^α are conjugate under the group.*

As there are exactly 5 groups of order 12 there are 9 cases in which there are exactly 5 groups of the given order g. In three of these cases g is not divisible by the square of a prime number, in one g is divisible by the cube of such a number, while in the remaining five, g is divisible by the square of

one and only one prime number. In one and only one of these cases g is completely determined, being 12. In another case g involves only two distinct prime factors, being $2p_1^2$. While in the remaining seven cases there is no upper limit to the number of distinct primes which may divide g when and only when it is odd. In fact, in five of these cases g must be odd while in the remaining two it may be either odd or even.

[1] Miller, Blichfeldt, Dickson, *Finite Groups*, 1916, p. 117.

ON INTERPOLATION TO HARMONIC FUNCTIONS BY HARMONIC POLYNOMIALS

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It is the object of the present note to indicate a certain easily proved result on interpolation to harmonic functions by harmonic polynomials, a result which the writer expects later to elaborate.

If the function $u(x, y)$ is harmonic in the circle C_R whose center is the origin and radius $R > 1$, and if $p_n(x, y)$ is the (unique) harmonic polynomial of degree n [that is, an expression of the form in polar coördinates $a_0 + a_1 r \cos\theta + b_1 r \sin\theta + a_2 r^2 \cos 2\theta + b_2 r^2 \sin 2\theta + \ldots + a_n r^n \cos n\theta + b_n r^n \sin n\theta$] which coincides with the function $u(x, y)$ in $2n + 1$ points equally spaced on the unit circle C, then the equation

$$u(x, y) = \lim_{n \to \infty} p_n(x, y) \tag{1}$$

is valid for (x, y) interior to C_R, uniformly for (x, y) on any closed point set interior to C_R. Moreover, if $u(x, y)$ has a singularity on the circle C_R, equation (1) is valid uniformly in no region containing in its interior a point of C_R.

Under the hypothesis of this theorem, if R_1 is chosen less than R but greater than zero, there exist harmonic polynomials $q_n(x, y)$ of respective degrees $n = 0, 1, 2, \ldots$ such that we have for (x, y) on C,

$$| u(x, y) - q_n(x, y) | \leqq \frac{M}{R_1^n},$$

where M depends on R_1 but not on (x, y) or n.[1] On the circle C the polynomials $p_n(x, y)$ and $q_n(x, y)$ are trigonometric polynomials of order n. From a general theorem due to Jackson[2] there follows immediately the inequality on C

$$| u(x, y) - p_n(x, y) | \leqq B \frac{M}{R_1^n} \log n,$$

where B is an absolute constant. If R_2 is chosen less than R_1 but greater than zero, the number M_1 exists such that we have

$$| u(x, y) - p_n(x, y) | \leqq \frac{M_1}{R_2^n} \tag{2}$$

for (x, y) on C. Consequently (Walsh, loc. cit.) the sequence $p_n(x, y)$ converges to $u(x, y)$ interior to the circle whose center is the origin and radius R_2, uniformly in any closed region interior to this circle. But R_2 (less than R and greater than zero) is entirely arbitrary, so the first part of the theorem follows. It will be noted that the inequality (2) cannot hold for even a single R_2 greater than R, in case $u(x, y)$ has a singularity on C_R.

The further remark, on convergence in a region containing in its interior a point of C_R, results directly from a theorem due to the present writer.[3]

The precise choice of the $2n + 1$ equally spaced points on C is immaterial and may vary with n; the points are conveniently but not necessarily chosen as the $(2n + 1)$st roots of unity. The uniqueness of the polynomial $p_n(x, y)$ as stated in the theorem refers naturally to uniqueness once the $2n + 1$ points themselves have been chosen, and this uniqueness is well known in the present case of trigonometric interpolation. The proof is now complete.

We have stated a result on interpolation by subdivision of C by an *odd* number of points; there is a precisely similar result, which the reader can prove by the same method, for equal subdivision of C by an *even* number of points. The two cases can be combined to yield a single sequence of harmonic polynomials, included in a single formulation, if desired. This method of formulation is indicated below in an analogous situation.

The theorem we have established is the exact analogue of a theorem on interpolation to analytic functions of a complex variable by polynomials in the complex variable:

If the function $f(z)$ is analytic in the circle $C_R: x^2 + y^2 = R^2 > 1$, and if $p_n(z)$ is the (unique) polynomial in z of degree n which coincides with the function $f(z)$ in the $(n + 1)$st roots of unity (or more generally in $n + 1$ arbitrary equally spaced points on the unit circle), then the equation

$$f(z) = \lim_{n \to \infty} p_n(z) \tag{1'}$$

is valid for z interior to C_R, uniformly for z on any closed point set interior to C_R. Moreover, if $f(z)$ has a singularity on C_R, equation (1') is valid in no region containing in its interior a point of C_R.

Interpolation in the $(n + 1)$st roots of unity has been studied by Runge[4] and Fejér,[5] who proved essentially the theorem just stated but without the determination of the exact region of uniform convergence.

The theorem we have established for interpolation by harmonic poly-

nomials can be expressed in a slightly different form. The function $u(x, y)$ is now supposed harmonic interior to the circle C_R (R not necessarily greater than unity), whose radius is R and center the origin, and the harmonic polynomial $p_n(x, y)$ of degree n is found by interpolation to $u(x, y)$ in $2n + 1$ points equally spaced on the circle C_r whose center is the origin and radius r less than R. The conclusion of the theorem is valid in the form previously stated. A limiting case of this revised theorem now suggests itself if we allow r to approach zero:

If the function $u(x, y)$ is harmonic in the circle C_R whose center is the origin and radius R, and if $\pi_n(x, y)$ is the (unique) harmonic polynomial of degree n defined by the requirement that $\pi_n(x, y)$ and all of its first, second ... nth partial derivatives at the origin shall be equal respectively to $u(x, y)$ and its corresponding first, second ... nth partial derivatives at the origin, then the equation

$$u(x, y) = \lim_{n \to \infty} \pi_n(x, y) \tag{3}$$

is valid for (x, y) interior to C_R, uniformly for (x, y) on any closed point set interior to C_R. Moreover, if $u(x, y)$ has a singularity on the circle C_R, equation (3) is valid uniformly in no region containing in its interior a point of C_R.

The conditions on $\pi_n(x, y)$ reduce in number to $2n + 1$ by virtue of the fact that both $u(x, y)$ and $\pi_n(x, y)$ are harmonic. The harmonic polynomial $\pi_n(x, y)$ is simply the sum of the first $2n + 1$ terms of the usual expansion of $u(x, y)$ in a series of harmonic polynomials (circular harmonics) in an arbitrary circle concentric with but interior to C_R. This identification, which yields the proof of the theorem, is conveniently made with the help of an analytical function $f(z)$ whose real part is $u(x, y)$, and of the Taylor expansion of $f(z)$ about the origin. The identification is left to the reader.

The function $u(x, y)$ is ordinarily considered to be real, but the theorem remains true if $u(x, y)$ is itself complex. In particular, if $u(x, y)$ is an analytic function of the complex variable $z = x + iy$, then the polynomial $\pi_n(x, y)$ is the sum of the first $n + 1$ terms of the Taylor development of $u(x, y)$ about the origin.

A new sequence of harmonic polynomials $\rho_n(x, y)$ of respective degrees $n + 1$ may be defined for interpolation to $u(x, y)$ by the requirement that $\rho_n(x, y)$ shall satisfy the restrictions (except that of degree) on $\pi_n(x, y)$ and in addition that we have

$$\frac{\partial^{n+1} \rho_n(x, y)}{\partial x^{n+1}}\Big]_{(0,0)} = \frac{\partial^{n+1} u(x, y)}{\partial x^{n+1}}\Big]_{(0,0)}, \quad \frac{\partial^{n+1} \rho_n(x, y)}{\partial x^n \partial y}\Big]_{(0,0)} = 0.$$

The conclusion follows for the sequence $\rho_n(x, y)$ precisely as stated for the sequence $\pi_n(x, y)$. Indeed, a single result is readily formulated which de-

scribes simultaneously the convergence of the $\pi_n(x, y)$ and the $\rho_n(x, y)$. Let $\sigma_m(x, y)$ be the linear combination of the first m of the functions

$$1, \ r\cos\theta, \ r\sin\theta, \ r^2\cos 2\theta, \ r^2\sin 2\theta, \ \ldots$$

which satisfies at the origin the first m of the conditions

$$\sigma_m = u, \ \frac{\partial\sigma_m}{\partial x} = \frac{\partial u}{\partial x}, \ \frac{\partial\sigma_m}{\partial y} = \frac{\partial u}{\partial y}, \ \frac{\partial^2\sigma_m}{\partial x^2} = \frac{\partial^2 u}{\partial x^2}, \ \frac{\partial^2\sigma_m}{\partial x\partial y} = \frac{\partial^2 u}{\partial x\partial y},$$
$$\frac{\partial^3\sigma_m}{\partial x^3} = \frac{\partial^3 u}{\partial x^3}, \ \frac{\partial^3\sigma_m}{\partial x^2\partial y} = \frac{\partial^3 u}{\partial x^2\partial y}, \ \frac{\partial^4\sigma_m}{\partial x^4} = \frac{\partial^4 u}{\partial x^4}, \ \ldots$$

Then $\sigma_m(x, y)$ is uniquely determined and is the polynomial $\pi_n(x, y)$ if $m = 2n + 1$ and is the polynomial $\rho_n(x, y)$ if $m = 2n + 2$. The sequence $\sigma_m(x, y)$ approaches $u(x, y)$ uniformly in any closed region interior to C_R, and if $u(x, y)$ has a singularity on C_R, the sequence $\sigma_m(x, y)$ converges uniformly in no region containing in its interior a point of C_R.

[1] Walsh, *Bull. Amer. Math. Soc.*, **33**, 591–598 (1927).
[2] *Theory of Approximation*, p. 121 (New York, 1930).
[3] *Trans. Amer. Math. Soc.*, **33**, 370–388, §9 (1931).
[4] *Theorie und Praxis der Reihen*, 126–142 (Leipzig, 1904).
[5] *Göttinger Nachrichten*, 319–331 (1918).

PARTIAL DIFFERENTIATION IN THE LARGE

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Communicated May 19, 1932

Two classical problems which to the best of our knowledge have never been solved are the following:

Problem 1.—Let $f(x, y)$ be a function of class C' on an open point set H. Let the partial derivative $f_{xy}\left(\frac{\partial}{\partial y}f_x\right)$ exist and have finite values on H. Then what can be said about the existence and values in the large of $f_{yx}\left(\frac{\partial}{\partial x}f_y\right)$?

Problem 2.—Let $f(x, y)$ be of class C' on an open set H and let all four second order partial derivatives

$$f_{xx}, \ f_{xy}, \ f_{yx}, \ f_{yy}$$

exist at each point of H.

What is the measure of the point set on which

$$f_{xy} = f_{yx}.$$

Problems of this type can be multiplied indefinitely, and a general theory of partial differentiation in the large to cover all such problems is desirable. We have started to construct such a theory. Among the various theorems which we have proved are three which we announce in this note as follows.

THEOREM 1. *Let $f(x, y)$ be of class C' on an open point set H. Let the four second partial derivatives*

$$f_{xx} \quad f_{xy} \quad f_{yx} \quad f_{yy}$$

exist everywhere on H. Then $f_{xy} = f_{yx}$ almost everywhere on H.

THEOREM 2. *Let $f(x, y)$ be of class C' on an open point set H. Let f_{xy} exist everywhere on H. Then there exists a point set W of positive measure on which f_{yx} exists and equals f_{xy}.*

THEOREM 3. *Let $f(x, y)$ be of class C' on the open point set H. At each point P of H let at least one of the two partial derivatives f_{xy}, f_{yx} exist. Then there exists a set W of positive measure on which both f_{xy} and f_{yx} exist and are equal.**

* For certain theorems on second order partial derivatives the reader is referred to the following articles:

Hobson, "Theory of Functions of a Real Variable," Cambridge, pp. 425, 426, 428 (1927).

Carathéodory, "Vorlesungen über reelle Funktionen," Berlin, 650 (1927).

EOCENE LAND MAMMALS ON THE PACIFIC COAST

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Read before the Academy Tuesday, November 17, 1931

The Sespe formation comprises a series of sandstones, shales and conglomerate, several thousand feet in thickness, and receives its name from the type locality of its occurrence on Sespe Creek north of the Santa Clara Valley, Ventura County, California. Originally described by Watts,[1] this formation has been recognized as furnishing an important stratigraphic record of the early Tertiary in the southern coast ranges with a position between marine sediments of Eocene age and marine sediments of Miocene age. At a number of localities in southern California deposits having the stratigraphic position and lithologic characteristics of the Sespe

are designated by that name and are regarded as the correlatives of this formation. Prevailing features which have been noted with regard to the Sespe are (1) the presence of red beds, and (2) the absence of a fossil record.

During the past few years a field study has been made of the Sespe with a view to recovering a paleontological record. Although the type section has yielded as yet no material, important fossil evidence has been found at several localities in adjacent regions.[2] It becomes increasingly apparent that the name Sespe, unless definitely restricted to a stratigraphic unit in the type section, loses its significance as a formational

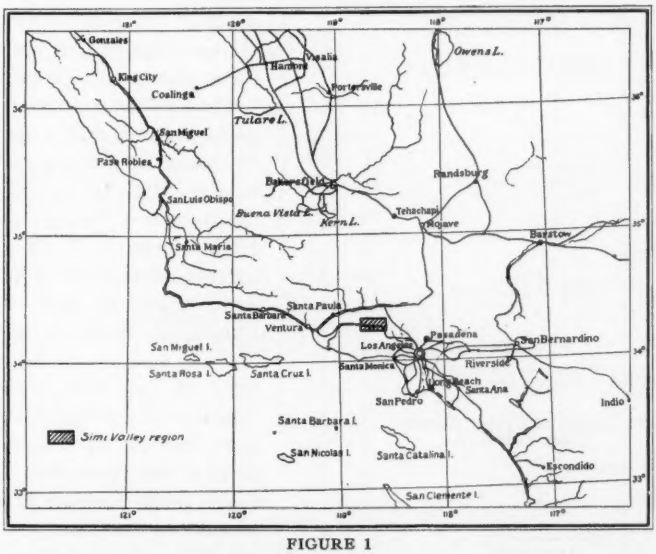


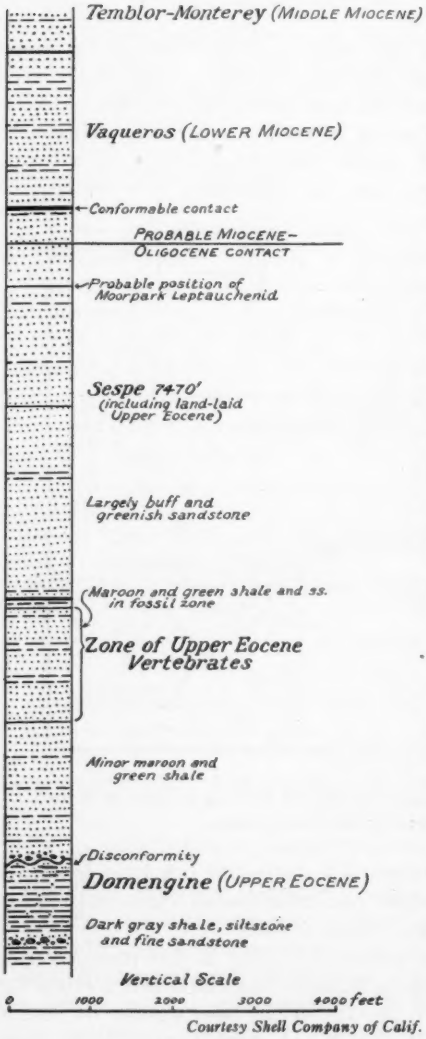
FIGURE 1

Outline map of a portion of southern California showing location of Sespe Eocene mammals in Simi Valley region.

designation and is expressive rather of a distinctive lithologic facies, for in deposits referred to the Sespe are vertebrate faunas which range in age from Eocene to upper Oligocene or lower Miocene.

Among the assemblages now known the oldest is that recorded from deposits exposed south of the Santa Suzanna Mountains and north of the Simi Valley, Ventura County. The region has been studied by several geologists and paleontologists, notably in recent years by W. S. W. Kew, by R. N. Nelson and by B. L. Clark. According to Kew,[3] who has mapped the area, the sequence of Tertiary formations includes marine Eocene, continental Sespe, marine Miocene and Pliocene. A very thick section

of sediments is exposed on the north limb of the Simi anticline immediately to the north of the Simi Valley (Fig. 1).


Temblor-Monterey (MIDDLE MIOCENE)
Vaqueros (LOWER MIOCENE)
Conformable contact
PROBABLE MIOCENE–OLIGOCENE CONTACT
Probable position of Moorpark Leptauchenid
Sespe 7470' (including land-laid Upper Eocene)
Largely buff and greenish sandstone
Maroon and green shale and ss. in fossil zone
Zone of Upper Eocene Vertebrates
Minor maroon and green shale
Disconformity
Domengine (UPPER EOCENE)
Dark gray shale, siltstone and fine sandstone
Vertical Scale
0 1000 2000 3000 4000 feet

Courtesy Shell Company of Calif.

FIGURE 2

A portion of the Tertiary stratigraphic column in the Brea Canyon section, north of the Simi Valley, Ventura County, California.

While the Eocene marine strata were regarded originally as belonging to the Tejon or upper division of this period, further study of the invertebrate faunas led Clark to recognize an earlier horizon, namely the Domengine (Upper Eocene). Immediately to the south of the Simi Valley, beds of questionable Tejon age occur above the Domengine.

Through the courtesy of the geological staff, Shell Company of California, I am permitted to give (Fig. 2) a portion of the measured Tertiary stratigraphic column in the Brea Canyon section north of the Simi Valley, as well as the lithologic characters of the Sespe as it occurs on the north limb of the Simi anticline. The deposits are here 7470 feet thick and may be conveniently divided into three parts. The lower Sespe consists of buff-weathering sandstones and conglomeratic sandstones with minor thicknesses of maroon and gray clays. The middle division is represented by maroon and green clays with fine-grained sandstones, alternating with coarse-grained buff and green sandstones. The middle Sespe, in contrast to the lower and upper divisions, is characterized particularly by variegated beds. The upper division consists in the main of

brown-weathering and gray sandstones and conglomerate. An erosion interval separates the lower beds from the marine Eocene (Domengine) while the upper Sespe grades without break into the Vaqueros marine deposits of lower Miocene age.

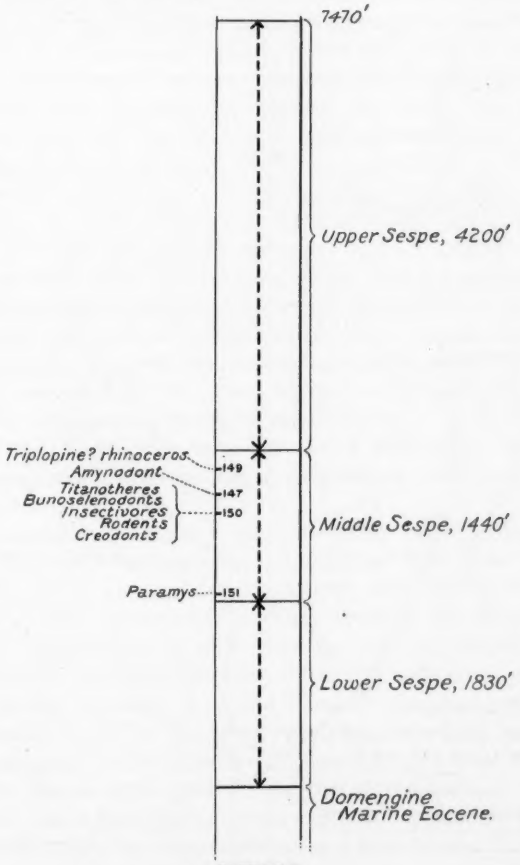


FIGURE 3

Stratigraphic column in Brea Canyon section showing thickness of principal divisions of the Sespe and vertical range of mammalian remains in middle Sespe (Calif. Inst. Tech. Vert. Pale. Localities 151, 150, 147 and 149).

Vertebrate remains have been found at several localities in the maroon and green clays and sandstones of the middle division. Stratigraphically, the occurrences range from approximately 1800 feet to 3000 feet above

the base of the Sespe (Fig. 3). The materials are fragmentary but the fauna as now known comprises at least fourteen different types.

The mammalian assemblage includes titanotheres, rhinoceroses of two types including an advanced amynodont and a small form related to either

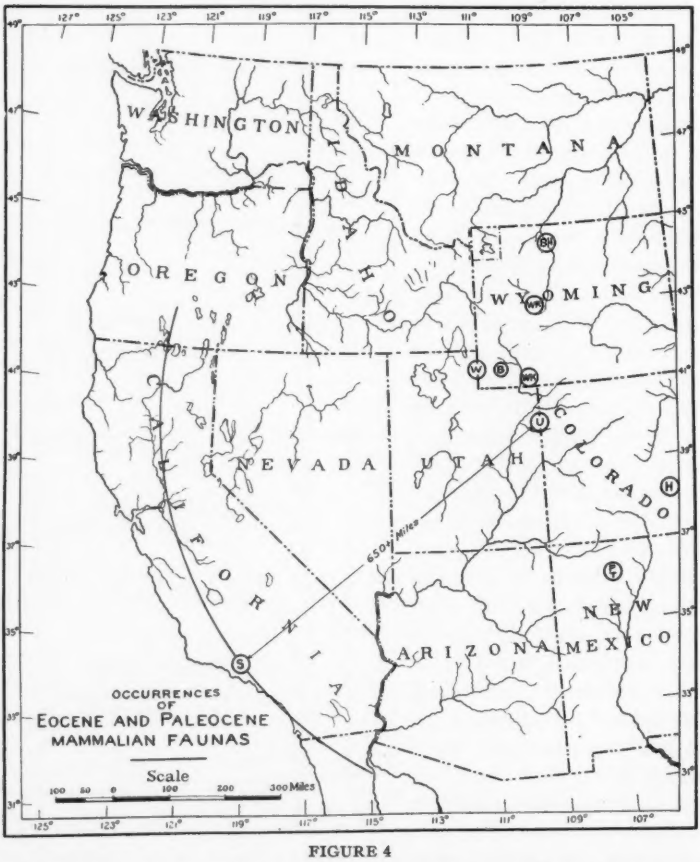


FIGURE 4

Outline map of western states and occurrences of Eocene and Paleocene faunas after Osborn. BH, Big Horn Basin; WR, Wind River; W, Wasatch; B, Bridger; WK, Washakie; U, Uinta; H, Huerfano; PT, Puerco-Torrejon. Newly discovered locality in the Simi Valley region indicated at S.

the triplopine or hyracodont group. Among the artiodactyls are *Protylopus* or a closely related form and an ancestral agriochoerid. Among the insectivores is a leptictid. Creodonta are represented by at least two

distinct members of the Hyaenodontidae and by a miacid. The rodents include the characteristic Eocene genus *Paramys* and a tiny representative of the cricetine group.

The most nearly related assemblages in time and in space are known to occur in the region of the Rocky Mountains and western Great Plains, 650 miles or more to the east of the Simi localities (Fig. 4). Preliminary study of this fauna, in the light of the known early Tertiary sequence of mammalian assemblages for western North America, suggests an age later than that of the Uinta Eocene (Uinta C) and earlier than that of the White River Oligocene. In this position the relationships appear to be closer to the Eocene than to the Oligocene assemblages. The presence of an amynodont and a triplopine rhinoceros, several distinct types of creodonts and the genus *Paramys* suggests an Eocene age. The titanotheres are known as yet only by isolated teeth. A number of incisors belonging to these types are characterized by rounded, reduced crowns, and in this respect the Simi forms are evidently more advanced than the Eocene genera *Dolichorhinus*, *Telmatherium* and *Protitanotherium*, and are more like types found in the Titanotherium zone of the White River. Among the creodonts is the genus *Hyaenodon*, generally regarded as characteristic of Oligocene faunas in North America. It is noteworthy, however, that the relatively large number of carnivores found at this stage comprises at least two families of the Creodonta with no representatives of the Carnivora vera.

A fauna exhibiting a similar stage of evolution has been described recently by Peterson[4] from deposits overlying the Upper Uinta (Uinta C) of the Uinta Basin. On the basis of relatively advanced types of titanotheres, provisionally referred to the genus *Teleodus*, the advanced character of the horses, tapiroids and cameloids, and the presence of a true *Hyaenodon*, Peterson assigns this stage to the basal Oligocene, designating it the Duchesne. Possibly the Sespe fauna of the Simi region, which resembles rather closely the Duchesne assemblage in stage of development, should be assigned likewise to the basal Oligocene. However, the number of distinctive Eocene types and the position of the fauna in the stratigraphic series above beds which do not represent apparently the highest Eocene marine strata as recognized in the Coast Ranges of California, have led me to regard the assemblage, at least for the present, as belonging to the uppermost Eocene.

[1] Watts, W. L., *Calif. State Min. Bur. Bull.*, **11**, 22–38 (1897).
[2] Stock, C., *Carnegie Inst. Wash. Publ.* **404**, Art. III (1930).
[3] Kew, W. S. W., *U. S. G. S. Bull. 691-M* (1919).
[4] Peterson, O. A., *Ann. Carnegie Mus.*, **21**, 61–78 (1931).